SUGAR PREFERENCES IN BIRDS: PHYSIOLOGICAL AND ECOLOGICAL CORRELATES

BY
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Some people seem able to work steadily. Fueled with an invisible drive, they seem to go on with no reassurance from outside. I am not one of these. For me, it was always pleasantly surprising to realize that somebody believed that my work had some promise, especially at times when I did not consider it a good bet. I thank the members of my committee (Karen Bjorndal, Jane Brockmann, Lincoln Brower, Peter Feinsinger, Lou Gillette, Brian McNab, and Bruce Stevens) for the encouragement and critical advice that made this work possible. I also thank them for letting me find my own way.

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

SUGAR PREFERENCES IN BIRDS: PHYSIOLOGICAL AND ECOLOGICAL CORRELATES

By

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The sugar preferences of birds seem to mirror the sugar composition of the rewards offered by plants. The nectar of plants pollinated by hummingbirds contains predominantly sucrose, whereas the pulp of bird-dispersed fruits contains mainly glucose and fructose and lacks sucrose. The hummingbirds Amazilia rutila, Cynanthus latirostris, and Chlorostilbon canivetii prefer sucrose over equicaloric solutions of glucose and fructose, whereas the frugivorous cedar waxwings (Bombycilla cedrorum) and European starlings (Sturnus vulgaris) prefer glucose and fructose over sucrose.

The preference of S. vulgaris and B. cedrorum for hexoses seems to be a consequence of the inability of these species to assimilate sucrose efficiently. Lack of intestinal sucrase activity and fast passage rates, respectively, are the physiological mechanisms responsible for this inefficient assimilation.

Among 22 avian species, 5 species belonging to the related families Muscicapidae, Mimidae and Sturnidae lack sucrase activity. For these birds, sucrose is a useless energy source that causes a feeding aversion. Lack of sucrase activity among birds is probably a phylogenetic constraint with profound behavioral and ecological implications.

Frugivores such as cedar waxwings, feed on fruits that contain seeds that have to be voided quickly. The rapid passage rates of frugivores apparently hinder the efficient digestion of sucrose, a disaccharide that must be hydrolyzed to be absorbed. Fast passage rates seem to link frugivory with the preference for hexoses. The absence of sucrose in fruit pulps is probably the result of diffuse coevolution between birds and plants and mediated by the digestive adaptations of birds to a fruit diet.

Hummingbirds assimilate sucrose, glucose and fructose with equally high efficiency (≥ 97%) and process sucrose and equicaloric hexose mixtures at similar rates. The preference of hummingbirds for sucrose over hexoses cannot be explained by differences in assimilation efficiency or digestive handling time.

GENERAL INTRODUCTION

> Standing on the shoulders of giants
> [I'd give] my kingdom for a voice
> M. Stipe (1988)

The reciprocal adaptations of plants and the animals that pollinate them and disperse their seeds are obvious and sometimes spectacular. These co-adaptations have fueled the imagination of many generations of natural historians (Baker 1979). This interest in mutualistic animal-plant interactions has produced an enormous amount of information about the sensory abilities, energetic needs, flight capabilities, and learning abilities of a variety of animals (Pearson 1953; von Frisch 1956; and Moermond and Denslow 1985 among others). My dissertation follows the interdisciplinary tradition of using the traits in plants to generate hypotheses and observations on the biology of their animal mutualists (Baker 1978). A perplexing pattern in the sugar composition of nectar and fruit pulp led me to explore the relationship between the digestive traits and the feeding choices of pollinators and seed dispersers.

The puzzling pattern that inspired my research was discovered by Herbert and Irene Baker. Baker and Baker (1982a, 1983) found that hummingbird-pollinated flowers tend to produce sucrose-rich nectars, whereas flowers pollinated by passerine perching-birds tend to be rich in hexoses. A similar

pattern occurs in fruits: small bird-dispersed fruits that are typically consumed by birds contain primarily hexoses, whereas fruits cultivated for human consumption tend to have high sucrose contents (Baker and Baker 1986, and pers. comm.). The ultimate goal of my research was to explain this differences in the sugar composition of the rewards that plants offer to different kinds of avian mutualists.

Baker and Baker not only provided the central question for my research, they also provided a strategy. "Presumably the offerings [made by plants] match the preferences of the birds, but to find why there should be such different preferences, more work by ornithologist/physiologists is needed" (Baker 1975, p. 3). This dissertation tackles the two challenges posed in Baker's statement. Do the preferences of different species of birds for simple sugars vary in a way that matches the sugar composition of nectar and fruit and, what are the physiological and ethological mechanisms that underlie these sugar preferences?

The first three chapters examine the sugar preferences in a variety of bird species and recognize the possible physiological and behavioral correlates of these preferences. In the remaining three chapters I evaluate the generality of some of the patterns revealed by the experimental results of the first three chapters (chapter 4), I synthesize these patterns in mathematical form (chapter 5); and I attempt to set them in an evolutionary context (chapter 6). Chapters are relatively self-contained and can be read independently. What

remains of this introduction provides broad outlines of the contents of each chapter.

In chapter 1 I describe the contrasting sugar preferences of three species of passerine omnivorous birds and identify one of the reasons why some birds reject sucrose. Some birds lack intestinal sucrase activity and therefore are unable to assimilate sucrose. The presence of undigested sucrose in the intestinal lumen has been shown to cause osmotic diarrhea and an associated conditioned aversion for sucrose (Suashine and Kretchmer 1964). Chapter 1 raises two questions which are addressed in subsequent chapters: How widespread is the absence of intestinal sucrase activity (chapter 4) and, what are the preferences of birds that possess intestinal sucrase activity (chapters 2 and 3)?

Chapter 2 is devoted to the sugar preferences of a relatively specialized frugivore that shows sucrase activity, the cedar waxwing (Bombycilla cedrorum, Martin et al. 1951). Cedar waxwings prefer glucose and fructose over sucrose. Although cedar waxwings show sucrase activity and are therefore capable of assimilating sucrose, they digest glucose and fructose more efficiently. I argue that their extremely rapid food passage rates hinder the efficient digestion and assimilation of a substrate, such as sucrose, that must be hydrolyzed before it is absorbed.

The sugar preferences of three hummingbird species are described in chapter 3. As would be expected from their sucrose-rich diets, hummingbirds prefer sucrose over glucose

and fructose. Hummingbirds, however, assimilate sucrose, glucose, and fructose with equally high efficiency (≥ 97%) and appear to process sucrose and equicaloric solutions of glucose and fructose at the same rate. Therefore, the sugar preferences of hummingbirds cannot be explained by differences in assimilation efficiency or digestive handling time.

In chapter 4 I conduct a comparative survey of intestinal sucrase activity among bird species. In a sample of 14 species of birds, sucrase activity varies over two orders of magnitude. Sucrase activity was from 2 to 118 times higher in hummingbirds than in the nine species of passerines that showed significant sucrase activity. Among seven families of birds, lack of intestinal sucrase activity seems to be restricted to a very well defined phylogenetic group: the sturnid-muscicapid lineage as defined by Sibley et al. (1988). Lack of intestinal sucrase in these birds seems to be an example of a phylogenetic constraint with profound behavioral and ecological consequences. The interspecific variation in sucrase activity is probably influenced both by feeding habits and phylogenetic affinities.

Chapters 1, 2, and 3 argue that sugar preferences are strongly influenced by relative rates of energy assimilation, and that these are the result of the interplay between sucrose hydrolysis, glucose and fructose uptake, and intestinal retention times. In Chapter 5 I integrate these variables in a mathematical model of sugar digestion. The

model uaas tha physiological traits of birds and tha sugar composition of food aa boundary conditions to predict optimal retention times, and to rank tha profitability of foods with different sugar composition. Current foraging thaory largely ignoras digestive constraints (Stephans and Krebs 1986). The model devalaped in chaptar 5 provides a tool to link tha digestiva procassas with the behavioral decisions of animals.

In chaptar 6 I attempt to put togather the nacessary alements for a plausible evolutionary explanation to tha pattarns in tha sugar constituants of nactar and fruit pulp. Chapter 6 explicitly states and avaluates tha stratagy that I usad throughout my rasaarch. This strategy was strongly influancad by tha methods usad by community acologists. Community acologists fraquantly approach acological problems in a multi-laval or "hlararchical" fashion (Diamond 1986). They attempt to idantify the behavioral mechanisms and popuiation processes that can account for broad acological pattarns (Grant, 1986). In chaptar 6 I usa this hlararchical approach to summariza tha rasults and conclusions of chaptars 1 to 5. I suggast that it is useful to look at tha distribution of sugars in nactar and fruit pulp as a broad ecological pattarn that can ba explained by behavioral procasaas (tha sugar prafarances of birds) and physiological and ethological mechanisms. In chapter 6 I also assass tha advantagas and limitations of this multi-lavel approach as a tool to provida avolutionary explanation for traits--such as the composition of sugar in nectar and frult--that may ba tha

result of historical coevolutionary processes.

CHAPTER 1
PHYSIOLOGICAL CORRELATES OF PREFERENCE AND AVERSION FOR SUGARS IN THREE SPECIES OF BIRDS

It has been commonly assumed that preference for substances perceived as sweet by humans is ubiquitous among vertebrates (Frings 1951). This preference, however, has been demonstrated experimentally for only a few species (Jacobs et al 1978). Another common assumption having little experimental support is that this preference is based on sweet taste (Hellekant 1979). Very different sugars are often considered equivalent, and preference for "sweet" substances is inferred from preference experiments using only one sugar (Brindley 1965; Hamrum 1953; Levey 1987a; Warren and Vince 1963). Some experimental evidence suggests that preference for simple sugars in vertebrates may not be so widespread (Jacobs et al. 1978). Preferences for simple sugars in many species may be mediated not only by taste but also by nutritional state and postingestional effects (Kare and Ficken 1963; Kare and Maller 1967; Shuford 1959, Tomljenovic-Borar 1967). Species showing a strong preference for one sugar may exhibit aversion to another (Schuler 1983).

Kare and Mason (1986) and Levey (1987a) have suggested that nectarivorous or frugivorous bird species are more likely to prefer sugars than granivorous or insectivorous species, and that sugar concentration and composition of fruits and nectar may influence fruit and flower choice

Presently these ideas cannot be substantiated because we know very little of the comparative biology of sugar preferences in birds. Except for Stiles' (1976) work on hummingbirds and Schuler's (1983) research on European Starlings (Sturnus vulgaris), few species have been tested with a variety of sugars. Especially relevant are the monosaccharidas glucose and fructose and the disaccharide sucrose contained in fruits and nectars (Baker and Baker 1983; Chan and Kwok, 1975).

Rogers and Muller (1973) showed that Red-winged Blackbirds (Agelaius phoeniceus) were indifferent to dilute sucrose solutions but rejected concentrated ones. These authors did not test preferences for monosaccharides. Schuler (1983) examined the preferences of S. vulgaris for sucrose and the monosaccharides glucose and fructose, and found that European Starlings strongly rejected sucrose solutions but avidly drank solutions of glucose and fructose. Why might both Red-winged Blackbirds and Starlings reject sucrose solutions? Absorption of the monosaccharides derived from sucrose (i.e. glucose and fructose) requires the hydrolysis of sucrose by the intestinal enzyme sucrase (Semenza 1968). Perhaps avian preference for sucrose is correlated with the presence or absence of sucrase activity. I examined the influence of sucrase activity on preferences of birds for simple sugars. I investigated the preferences of three species of omnivorous birds (European Starlings, Red-winged Blackbirds and Common Grackles (Quiscalus quiscula)) for the disaccharide sucrose, and for its constituent monosaccharides

(glucose and fructose) I then tested the hypothesis that sucrose averse birds were sucrase deficient by measuring blood glucose changes after a sucrose or a monosaccharide meal, and by quantifying the activity of intestinal sucrase in preparations of intestinal mucosa I predicted that intestinal sucrase activity in the three species examined would be ranked in the same order as preference for sucrose

Methods

Test birds were captured at the Denver Animal Damage Research Center, Gainesville, Florida, at least three months before the experiments and acclimatized in 1 3 X 1 3 X 2 m outdoor cages at a density of 25 birds per cage Processed chicken feed (Purina Layeena) and water were provided ad libitum during that period During experiments, birds were housed in a covered aviary under natural temperature and photoperiod conditions Each bird was caged individually (cage size 48 X 48 X 72 cm) and isolated visually A 5 day acclimation period during which feed and water were presented ad libitum preceded each experimental trial Adult males and females of S. vulgaris and Q quiscula and adult males of A phoeniceus were used in the experiments I conducted all experiments during July and August 1986 at the facilities of the Denver Wildlife Research Center, Gainesville Field Station

Preference Tests

Solutions were prepared 12 to 24 hours before the experiments and presented to the birds in closed glass drinking tubes (50 cm long, internal diameter 1 1 mm). The tubes consisted of a 42 cm vertical section, and a lower 7 cm section that was bent upward at a 45° angle, an elliptical hole (2 X 1 5 cm) in the top wall of this last section provided access to the solutions.

Each trial spanned two days On day 1 birds were trained to use the drinking tubes by exposing birds in sequence to a tube containing the test solution for 1 hr and then for another hour to a tube containing distilled water Test solution and water drinking tubes were marked with different colored tapes wrapped just above the drinking holes Color was used because a preliminary set of experiments suggested that the birds had difficulty distinguishing between solutions and water when only positional (left and right) cues were given (see also Schuler 1983) On day 2, test solution and water tubes were presented simultaneously but separated by 10 cm. Colors were alternated systematically between left and right to minimize positional bias After each trial, consumption was measured to the nearest millimeter and subsequently transformed to volume units Food and water were removed from the cages during trials

I used a completely randomized factorial design (2 X 3) with 2 colors and 3 osmolarities of test solutions (0.175 M, 0.35 M and 0.7 M) in all experiments Eight birds were

randomly assigned to each cell (color and concentration combination). The variable used for preference was the amount of test solution consumed divided by total fluid consumption

A two-way ANOVA was conducted on preference values to determine interactions between color and concentration. If no significant interactions were found ($p > 0.05$), the null hypothesis that preference at each concentration was not significantly different from 0.5 (indifference point) was tested using multiple contrasts (Chew 1976; Kirk 1982).

I conducted two experiments with each species. Experiment 1 tested preference for mixtures of glucose and fructose (1:1 ratio) against water; red and green were used to mark the drinking tubes in this experiment. Experiment 2 tested preference for sucrose against water, and white and blue were used to mark the drinking tubes.

Blood Tests

Fifteen birds of each species were used in each test. The birds were fasted overnight and randomly assigned to five groups of three individuals. In one group, birds were sham-intubated while the rest were gavaged with 3 g per Kg of bird mass of a 1:1 mixture of glucose and fructose administered in a 1.66 M solution. Blood was obtained from the sham-intubated birds 1 minute after intubation. Blood was obtained from birds in each of the remaining groups at 15, 30, 60 or 90 minutes after intubation. Blood collection and treatment followed Cohen (1966). Samples were centrifuged for 3 minutes

in an International Micro-capillary Centifuge, plasma was separated from cellular elements, and blood glucose concentration (BGC) was measured using a glucose oxidase method (Worthington Statzyme, Cooper Biomedical)

After 10 days I randomly re-assigned the same birds to five groups of 3 individuals and the same procedure was repeated using a 0 87 M solution of sucrose

Sucrase Activity

Birds were anesthesized with metaphane The small intestines were excised, chilled in ice cold 1 02% NaCl and scraped of mucosa using a glass slide Mucosal samples for each bird were taken at proximal, medial and distal sections of the small intestine Mucosal scrapings were homogenized (0 3-0 5 g in 6 ml of 1 02% NaCl) using a Brinkman Polytron homogenizer (25 sec at setting # 6) and the resulting suspensions were stored in liquid nitrogen Just prior to sucrase assays, the homogenates were thawed at 22°C, and kept on ice. Protein concentration of homogenates was measured using the Bio-Rad kit (Bio-Rad, Richmond, CA), with gamma globulin standard

I assayed sucrase activities with a colorimetric method modified from Dahlqvist (1964) and Trinder (1969). Briefly, the reaction was started by adding 33 µl of thawed mucosal homogenate plus 33 µl of sucrose stock (0 056 M sucrose in 0.1 M sodium malate buffer PH 6.0). After incubating at 38°C for 10 min, then 1 ml of stop/develop reagent stock was added

to arrest the sucrase activity and to measure liberated glucose. My stop/davalop raagant waa madm by disaolving 1 bottle of "Glucose (Trinder)-315-100" reagent powdar (proprietary mixture of Sigma Chemical Co.) plus 50 ml of 1.0 M Tris/HCl (pH 7.0) and 50 ml of 0.5 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.0). Blanks were prepared by separataly incubating 33 µl of mucosal homoganata at 39°C for 10 min, after which time 33 µl of sucrose stock plus 1 ml of stop/develop reagent was addad. Tha arreatad reactiona were then allowed to stand at 22°C for 18 min, at which time the absorbances were measured at 505 nm using a Backman DU-7HS digital spectrophotometer. Glucose standards (0 to 40 µg in 66 µl of 0.1 M aodium malaata buffer pH 6.0) ware also simlarly raacted with tha atop/devalop reagent. Based on absorbanca maasurementa, sucrase actlvitias wera subsaquantly calculatad as µmole/min mg protain. Ia prallmiaary taats of the assay I found that sucrose hydrolysis increased linmerly for absorbances lower than 0.8. Incubation timas of 10 minutaa conaistently gave absorbance valaes in the linaar ranga.

Results

Preference Tests

Wa found no aignificant interactions batween concentration and color in any of the experiments ($0.09 \leq F \leq 2.63$, $df = 2, 47$, $p > 0.05$). All apecias aignificantly preferred aolutiona containing glucose and fructose at 0.35 M

and 0.7 M concentrations (Fig 1-1), but were indifferent to 0.175 M solutions.

Starlings significantly rejected the most concentrated of the sucrose solutions (0.7 M) and were indifferent to 0.175 M and 0.35 M solutions (Fig. 1-1). Regardless of concentration the amount of sucrose solution consumed in day 2 was significantly less than the amount consumed on day 1 ($p < 0.01$, sign test Fig 1-2). In contrast consumption of glucose + fructose solutions by S. vulgaris increased significantly for the most concentrated solution (0.7 M) from day 1 to day 2 ($p < 0.05$, sign test), and was not significantly different for 0.175 M and 0.35 M solutions (Fig. 1-2).

Red-winged Blackbirds were indifferent to 0.175 M solutions of sucrose, significantly preferred 0.35 M solutions and rejected 0.7 M solutions (Fig 1-1), showing a typical indifference-preference-aversion curve (Herriman and Kare 1966). This result contrasts with Rogers and Maller's (1973) report of a continuously decreasing preference-concentration curve. Common Grackles significantly preferred sucrose solutions over water at all concentrations (Fig 1-1).

Blood Tests

A sharp increase in BGC followed the administration of the glucose-fructose mixture in all species. BGC peaked at about 30 minutes after intubation and declined thereafter

(Fig. 1-3). Red-winged Blackbirds and Common grackles showed qualitativaly similar BGC curves after a sucrose load (Fig 1-3). These curves are only slightly lower than the ones obtained after a glucose and fructose load. In Starlings no elavation in BGC was found after administration of sucrose

Enzyme Activity

We measured how sucrose enzymatic hydrolysis varied with position along the small intestine in 3 individuals of each species (Fig. 1-4). Sucrose hydrolysis per unit milligram of protein decreased distally in Common Grackles but remained relatively constant in Red-winged Blackbirds. Sucrase activity was consistantly higher in Common Grackles than in Red-winged Blackbirds. I was unable to detect any sucrase activity in Starlings

Discussion

This study demonstrated that interspecific differences in preferances for sucrose occur among birds and that these preferances are correlated with relative sucrase activity The three species studied can be ranked with regard to these preferances as sucrose prone (preferring sucrose to water at all concentrations tested, Common Grackles), sucrose tolerant (preferring intermediate concentrations of sucrose over water but rejecting concentrated ones, Red-winged Blackbirds), and sucrose intolerant (rejecting concentrated solutions and developing a conditioned aversion to even diluted

concentrations, Starlings). Sucrase activity was, as predicted, ranked in the same order as preference for sucrose solutions, with Common Grackles showing the highest activity, Red-winged Blackbirds intermediate levels and Starlings lacking detectable activity. In animals with disaccharidase deficiencies, the ingestion of substrates is accompanied by both osmotic and fermentative diarrhea (McMichael et al. 1965, Sunshine and Kretchmer 1964). The development of an aversion for sucrose solutions shown by Starlings is probably a result of the effects caused in the intestine by a substrate that can be neither digested nor absorbed.

In contrast to the results reported by Rogers and Mallar (1973), I did not find that preference decreased as sucrose concentration increased. Instead, Red-winged Blackbirds were indifferent to sucrose solutions at low concentrations, significantly preferred 0.35 M solutions over water, and rejected concentrated solutions. The discrepancy between my results on Red-winged Blackbirds and those reported previously by Rogers and Mallar (1973) may be due to differences in the source populations or differences in diets during the acclimation period before the experiments. Rogers and Mallar's birds were captured in Maryland, whereas the birds I used were captured near Gainesville, Florida. It is conceivable that there is interpopulation variation in preference resulting from variation in diets. Rogers and Mallar fed their birds Purina Game Bird Flight Conditioner (PGBFC), while I fed ours Purina Layeena (PL). PGBFC is a

high protein diet (>30% crude protein) and does not include molasses products (containing sucrose) in its composition, whereas PL contains less protein (> 16%) and includes molasses products (data from the feed bags). Rosensweig et al. (1968) and Stifal et al (1968) have shown that dietary levels of carbohydrates result in changes in jejunal glycolytic enzymes in humans and rats, and Karasov et al (1983) showed that a high carbohydrate diet induces intestinal monosaccharide transport. It is very likely that the adaptive processes taking place whenever an animal changes its diet will result in preference changes. However, since all the birds used in my experiments were fed exactly the same diet for an extended period of time, the interspecific enzyme activity differences found are not just physiological adaptations to a particular diet but probably possess a strong genotypic component.

Hanson and Parsons (1976) and Riesenfeld et al (1980) have shown in the Chicken (Gallus gallo) that part of the glucose absorbed after a meal is converted to lactate in the intestinal wall. The observed increase in BGC after a glucose-containing meal, therefore, is not an absolute indicator of the amount of glucose that is absorbed, and the technique is only useful for comparative purposes. As shown by the data, it serves only to distinguish sucrose digestors (species or individuals) from non-digestors (see Newcomer et al. 1975 for the performance of a similar technique used to detect lactase deficiency in humans).

The variations in sucrase activity found among European Starlings, Common Grackles and Red-winged Blackbirds may be correlated with their feeding habits in nature. Although the three species used in the experiments are largely omnivorous, the proportions of insects, grain and fruit they eat vary widely among them. Common Grackles and Red-winged Blackbirds include large amounts of grains and seeds in their diets, especially during the winter and fall (Beal 1900; Martin et al. 1951), whereas European Starlings are much less granivorous and have insects and fruit as the main components of their diets, consuming only small amounts of grain or grain products during the winter (Kalmbach and Gabrielson 1921, Martin et al. 1951). Starlings show lower levels of activity of other intestinal carbohydrases such as maltase and isomaltase than the other two species (Martinez del Rio and Stevens 1989), and have been shown to fare poorly on high carbohydrate grain diets (Veitt 1973, Thompson and Grant 1968). Berthold (1976) has suggested that many temperate bird species use fruit as a nutritional supplement to either insects or grain (see also Martin et al. 1951). The pulp of most bird dispersed fruits is rich in glucose and fructose but contains only small amounts of sucrose (whereas fruits used for human consumption tend to be richer in sucrose, Baker and Baker 1986). Starlings can include many species of fruits in their diets because fruits either lack sucrose or contain only small amounts of it (chapter 6). By acting as discriminating consumers, birds may have influenced in

evolutionary time the sugar composition of the fruits they consume and whose seeds they disperse. The preferences of fruit eating birds for different sugars and the distribution of sucrase among them may have important coevolutionary consequences (chapter 6).

The distribution of disaccharidases among mammals and birds is extremely heterogeneous (see Semenza, 1968, and Vonk and Western, 1984). Unfortunately too few species have been assayed to establish a clear relationship between dietary habits and the activity of various carbohydrases. Data suggest, however, that the activities of specific enzymes are reduced when their substrates are rare or absent from an animal's diet (Vonk and Western, 1984). My results indicate that variations in sucrase activity, presumably resulting from genotypic adaptations to dietary differences, are correlated with the preference of birds for sucrose. Maybe insectivorous and carnivorous birds have low levels of sucrase and, therefore, are sucrose averse. Granivorous birds, in contrast, probably have higher sucrase activities and are sucrose tolerant or sucrose prone. In chapter 4 I will critically examine the ecological and phylogenetic correlates of sucrase activity in birds.

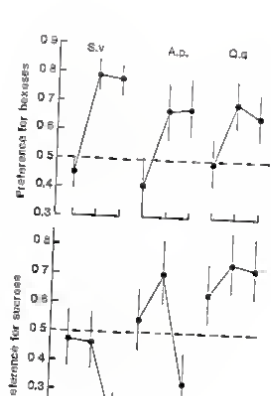

Fig 1-1.- Comparative preferences for a mixture of glucose and fructose (above), or sucrose (below) against water. Bars are 95% confidence intervals and the dashed line at 0.5 indicates no preference. S.v. is Sturnus vulgaris, A.p. is Agelaius phoeniceus, and Q.q. is Quiscalus quiscula.

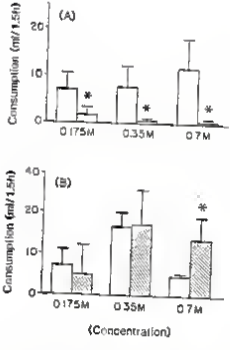

Fig 1-2 - S. vulgaris changes in consumption of sucrose (a) or a mixture of glucose and fructose (b) from day 1 (open columns) in which solutions were presented singly and day 2 (stipled columns) in which they were presented paired with water. Bars are standard errors. Note that there was a decrease in consumption of sucrose from day 1 to day 2, but that the consumption of the glucose and fructose either remained equal or increased. Asterisks indicate a significant change in test solution consumption from day 1 to day 2 ($p < 0.05$, sign tests)

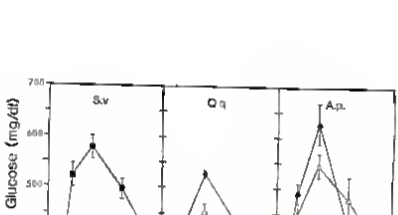

Fig 1-3 - Temporal changes in blood glucose concentration after intubation with monosaccharides (closed) or sucrose (open) for S. vulgaris (S-v), Q. quiscula (Q-q) and A. phoeniceus (A-p) Bars are standard errors

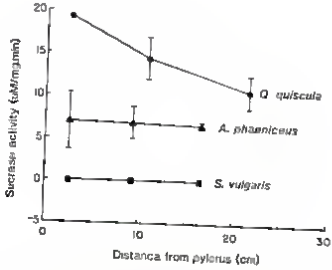

Fig. 1-4 - Sucrase activity as a function of intestinal position in three species of birds. Activity is normalized to mg of mucosal protein. Points are means and bars are standard errors.

CHAPTER 2
PHYSIOLOGICAL BASIS AND ECOLOGICAL CONSEQUENCES OF SUGAR PREFERENCES IN CEDAR WAXWINGS

The disaccharide sucrose and the monosaccharides hexoses, fructose and glucose, are the most common simple sugars in fruit pulp and nectar (Chan and Kwok 1975, Baid and Freeman 1986; Baker and Baker 1983, 1986). Hummingbird-pollinated plants produce nectars with a high proportion of sucrose whereas passerine-pollinated plants produce nectars with a low proportion of sucrose and high proportion of hexoses (Cruden and Toledo 1977; Baker and Baker 1982, 1983; Scogin and Freeman 1984). Small bird-dispersed fruits, which are typically fed upon by passerines, contain mainly hexoses, whereas cultivated fruits used for human consumption tend to have high sucrose contents (Baker and Baker 1986, and pers. comm.). These differences in sugar composition remain largely unexplained. It has been postulated that chemical characteristics of nectar and fruit pulp evolved in response to the preferences of pollinators and seed dispersers (Baker and Hurd 1968; Howell 1979, Levey 1987). With few exceptions (Hainsworth and Wolf 1976; Stiles 1976), the sugar preferences of birds that feed on nectar and fruit are unknown.

Glucose, fructose, and sucrose have different modes of intestinal transport and produce different metabolic effects

after ingestion and absorption (Sestoft 1981, Gray 1981). Even though they are similar chemically and in caloric value, these sweet substances may not be biologically equivalent. Schuler (1983) has demonstrated that the European Starling (Sturnus vulgaris) rejects sucrose solutions but readily accepts solutions of fructose and glucose. Sucrose aversion in the Starling is associated with a deficiency in the intestinal enzyme sucrase which hydrolyzes sucrose into fructose and glucose (chapter 1). Animals lacking sucrase are unable to digest and absorb sucrose; in consequence, the presence of undigested sucrose in the intestine of these animals can cause severe osmotic diarrhea. In this way, the absence of intestinal sucrase can be associated with aversion for sucrose (Sunshine and Kretchmer 1964).

In this chapter, I report the sugar preferences of the Cedar Waxwing (Bombycilla cedrorum), one of the most heavily frugivorous birds in temperate North America (Martin et al. 1951). I also analyze the influence of taste and postingestional factors on these preferences. Since most bird-dispersed fruits in temperate North America are hexose dominated (I. Baker and H. G. Baker in litt.), I predicted that Cedar Waxwings would prefer glucose and fructose over sucrose. I also hypothesized that this preference would be a result of sucrase deficiency. I examined these hypotheses by using simple tests in which birds were offered a choice between sugars; by estimating the efficiency with which the birds digested and absorbed sucrose, glucose and fructose in

vivo, and by assaying for sucrase in preparations of intestinal mucosa.

Methods

Ten Cedar Waxwings were captured in Madison, Wisconsin, during October 1985. Birds were kept indoors and housed together (cage size: 2 X 2 X 1 m) for approximately two weeks, after which I transferred them to individual cages (40 X 55 X 55 cm). They were fed a diet of mashed bananas and soy protein isolate ad-libitum ('banana mash') and provided with water. The composition of simple sugars in this diet was 59% glucose, 38% fructose and 3% sucrose (sugar analysis performed by I. Baker). The aviary had a 12D-12N hrs light cycle and constant temperature of 23° C (± 1° C).

Choice Experiments

Sugars were presented to the birds in cubes made with 10 grams of agar (Difco Bacto-Agar), 150 grams of sugar (reagent grade), 2 ml of red food-dye (McCormick & Co., Inc.) and 1000 grams of boiling water. The resulting gel was cut into small cubes (about 0.6 X 0.6 X 0.6 cm and 0.18 grams, SD = 0.06 g, N = 50). The concentration of sugar used is within the range of concentrations encountered by birds in fruit (Moermond and Denslow 1985, White and Stiles 1985). Except where stated, sugar concentrations in the cubes are expressed as g sugar/100 g water. The cubes were presented in two plastic trays (5 X 3 X 3 cm) each containing cubes with one kind of sugar

cube. Trays were attached to each side of a perch. The amount eaten from each tray was estimated as the difference between the weight of the tray before and after each experiment. To estimate weight loss resulting from evaporation of cubes, two trays containing the same sugars used in the experiment were weighed, left inside the aviary for the duration of the trial and weighed again at the end. Since loss by evaporation was minimal (mean amount lost by evaporation ± SD = 0.4 ± 0.2 g, N=20, about 3% of the total amount), I used uncorrected weight values for analysis. I performed all choice experiments in the aviary about 30 minutes after the lights were switched on (ca. 0800). Water was provided during the trials, but the banana maintenance diet was removed.

I performed two series of choice tests with ten birds. In series 1 I tested preferences for glucose, fructose, sucrose and a mixture of glucose and fructose (1:1 ratio) by offering the birds a choice between a tray containing agar cubes with sugar ('sugar' cubes) and a tray containing agar cubes made without sugar ('pure agar' cubes, 10 g of agar, 2 ml of food dye and 1000 g of water). Preference for each sugar was calculated by dividing the weight of sugar cubes eaten by the total weight of both sugar and pure agar cubes that were eaten (Kare et al. 1957). To determine whether the birds could taste sugars, I observed 2-4 birds in each trial and recorded the number of cubes swallowed or dropped.

In series 2 I compared preferences between sugars by presenting each bird with a choice between two trays, each

containing agar cubes made with a different sugar or mixture of sugars. All possible pair-wise combinations of glucose, fructose, sucrose and the mixture of glucose and fructose were tested. The position of the trays (left or right) was randomized for each bird in every experiment. In each preference test I tested the null hypothesis that preference, as defined above, was not significantly different from 0.5 (the indifference point where consumption of both sugars tested is equal) using one sample t-tests on arcsine$\sqrt{}$ transformed preference values. All trials lasted 1.5 hrs, with the exception of an experiment in which sucrose was paired with fructose. This experiment was repeated using both 1.5 and 3 hrs long trials. The same ten birds were used in all the preference experiments. I collected excreta samples from each bird after every experiment and measured their sugar concentration with a pocket refractometer. Although urates and other fecal contaminants with undetermined refractive properties make the refractometer readings imprecise (Inouye et al. 1980), I found different sugar concentrations in excreta of birds fed on different sugars.

Extraction Efficiency and Retention Time

To measure the efficiency with which cedar waxwings absorbed each sugar, I used a double-isotope method (Karasov et al. 1986). Agar cubes were prepared using $^{14}C(U)$ (uniformly labeled) sugar (D-glucose, fructose, or sucrose) and ^{3}H polyethylene glycol (PEG, molecular weight 4,000) as

an inert marker that is not absorbed in the gut. Each 10 gram cube contained 20 microcuries of ^{14}C(U) sugar and 75 microcuries of ^{3}H PEG. In each experiment six birds were deprived of food for 15 minutes and then allowed to feed on these labeled cubes for 2 minutes. The mean weight of radioactively labeled cubes eaten per bird was 0.23 g (SD = 0.06 g). After this period I allowed birds to eat banana mash *ad-libitum*. Excreta were collected from plastic sheets at the cage bottoms at 15, 30, 45, 75, 135, 195 and 255 minutes. After 255 minutes counts of ^{3}H PEG in excreta were less than 4 times background. Recovery of the PEG marker ranged from 72 to 96 percent (mean ± SD% = 0.82 ± 0.09, N = 18). Excreta were counted for ^{3}H and ^{14}C using double-isotope procedures (see Karasov and Diamond 1983 for details). Extraction efficiency was calculated by the inert-indicator ratio method (Kotb and Luckey 1972, Karasov et al. 1986) as

$$100 - 100[(cpm_{PEG}/cpm_{sugar})_{food} \times (cpm_{sugar}/cpm_{PEG})_{excreta}],$$

where cpm is counts per minute in a scintillation counter.

Because all ingested PEG is excreted, an extraction efficiency of 100% is obtained if $(cpm_{sugar})_{excreta}$ equals zero, and an efficiency of 0 is obtained if the ratio cpm_{sugar}/cpm_{PEG} has the same value in excreta as in food. The quantity defined here as extraction efficiency is a synonym for apparent digestibility (Church and Pond 1982).

I obtained mean retention times for PEG by fitting the cumulative excretion as a function of time to the equation

$$Y(t) = 1-\exp(-k(T_t-t)) ,$$

with a nonlinear least squares program (SAS procedure NLIN, SAS Institute). Y(t) is the cumulative proportion of marker after time t, T_L is the transit time for the leading edge of the bolus, and k is the rate constant for excretion. An estimate of the mean residence time of marker particles in the digestive tract was estimated by summing the estimated value of the reciprocal of k with the estimated value of T_L (Karasov et al. 1986; Fenry and Jumars 1987). The reciprocal of the mean residence time is a measure of food passage rate (i.e., low mean residence times imply high passage rates). Six birds were used in this experiment.

Sucrase Activity

The methods used to measure intestinal sucrase activity are outlined in detail in chapter 1.

Results

Choice Experiments

Series 1. Birds strongly preferred sugar cubes over pure agar cubes (Table 2-1). Contary to expectations, total consumption of cubes (sugar+pure agar cubes) was significantly higher for sucrose than for all the other sugars (sign tests, $p < 0.01$ in all cases, Table 2-1). The percentage of sugar in excreta was also significantly higher for sucrose than for all other sugars (sign tests, $p < 0.01$ in all cases, Table 2-1), suggesting a lower sucrose digestibility.

The birds picked up individual cubes and manipulated them in their bills, repeatedly touching the cubes with their tongues and then either swallowing or dropping them. Sugar cubes were almost always swallowed after having been picked from the tray whereas pure cubes were almost always dropped (Table 2-2).

Series 2. When given a choice between sugars, Cedar Waxwings significantly preferred glucose and the mixture of glucose and fructose over both fructose and sucrose (Table 2-3). Glucose was not significantly preferred over the mixture. In a 1.5 h trial the birds appeared to show no preference between fructose and sucrose. When a longer trial (3 h) was used, however, the birds significantly preferred fructose over sucrose (preference for fructose = 0.59 ± 0.13, $t = 2.31$, $p < 0.05$). The ranking of preferences therefore is: glucose = glucose + fructose > fructose > sucrose. As predicted, Cedar Waxwings preferred monosaccharides over sucrose.

In all tests involving sucrose versus monosaccharides the amount of sugar in excreta decreased significantly as the preference for monosaccharides increased (Fig. 2-1). This negative correlation suggests that birds preferring sucrose were assimilating less than birds preferring monosaccharides. The total amount of cubes eaten was significantly higher in those birds preferring sucrose over monosaccharides, the total amount of cubes consumed in each trial was positively correlated with preference for sucrose (Fig. 2-2).

Extraction Efficiencies

Extraction efficiencies by the birds for both glucose and fructose were significantly higher than that of sucrose (paired t-tests, $t = 6.28$, $t = 4.51$ respectively, $p < 0.05$, Table 4-2). The extraction efficiency of glucose appears higher than that of fructose, although the significance is marginal ($t = 2.52$, $p = 0.053$, Table 2-4). Extraction efficiencies were, thus, ranked in the same order as preferences. I had hypothesized that Cedar Waxwings would be sucrase deficient, and therefore unable to digest sucrose. This hypothesis was falsified by the estimated extraction efficiency found for sucrose; Cedar Waxwings were able to digest sucrose albeit less efficiently than glucose or fructose.

The averages of mean residence times of PEG, and thus of water soluble food particles in the gut, were 41.5 min (± SD = 4.2, N=6) for the glucose experiment, 41.3 min (± 6.5, N=6) for the fructose experiment and 39.1 min for the sucrose experiment (± SD = 6.2, N=6). PEG excretion per interval and cumulative excretion are shown in Fig. 2-3. The frequency distribution of excreted particles was a decreasing function of time and was adequately described by exponential density functions (the coefficient of correlation for individual trials ranged from 0.84 to 0.95, Fig. 2-3).

Sucrase Activity

Because the extraction efficiency experiments indicated that Cedar Waxwings were capable of digesting sucrose, it was not surprising to find sucrase activity in the mucosal preparations. The affinity of this enzyme for sucrose as measured by K_m did not vary appreciably among the three birds examined or among the intestinal segments (Mean K_m=15.5 mM, ranging from 15.2-15.7 mM). The maximal rate of sucrose hydrolysis (V_{max}) decreased from the proximal to the distal segments of the intestine regardless of whether sucrase activity was normalized to protein concentration or tissue length (Fig. 2-4). Sucrose hydrolysis increased with concentration in a typically decelerating fashion. Figure 2-5 summarizes our results on the kinetics of sucrase activity.

Discussion

Many previous studies have shown that birds can detect sugars (Lavey 1987 and references included), but few have shown that taste is involved in sugar detection (Kare and Mason 1986). Cedar Waxwings were able to distinguish between cubes with and without sugars before swallowing them. Taste, therefore, is probably involved in their recognition of sugars. In short term trials (1.5 h), Cedar Waxwings were also able to discriminate among different sugars. The cues used by birds to achieve this are unknown. Taste may have been important, but position and the texture of the cubes made with different sugars may also have been involved. Jacobs

et al. (1978) have suggested that flavors, as well as other cues, serve as markers for what animals have learned about food items (see also Brower, 1984).

Total consumption of cubes was higher in the sucrose vs pure agar trial than when hexoses were paired with pure agar, and preference for sucrose over monosaccharides was positively correlated with total consumption of cubes. These results indicate that birds feeding on sucrose or preferentially on sucrose were more stimulated to eat. Since sucrose is absorbed less efficiently than either glucose or fructose, the birds had to eat more to increase their absorbed energy intake.

Blood glucose level and its monitoring by brain glucoreceptors have been implicated in many theories of control of food intake (Le Magnen 1985). High levels of blood glucose are usually associated with lowered food intake. The rate at which circulating glucose increases in Cedar Waxwings is probably higher after a glucose (or a glucose + fructose) meal than after a sucrose meal of equal size. Glucose and glucose+ fructose meals are probably more efficient at suppressing the feeding response ('hunger') than sucrose and are therefore preferred.

The difference in absorption efficiency between glucose and fructose was relatively small. Why then did Cedar Waxwings strongly prefer glucose and glucose + fructose over fructose? Rowland and Stricker (1979) have shown that intravenous infusions of fructose are less efficient than

equicaloric infusions of glucose in suppressing insulin-induced feeding in rats. Fructose cannot cross the blood-brain barrier in mammals (Oldendorf 1971) and its effect in suppressing hunger may be less than that of glucose both in rats and in Cedar Waxwings. This hypothesis is supported by the observation that in 1.5 hours trials Cedar Waxwings did not prefer fructose over sucrose in spite of the fact that fructose was absorbed more efficientiy in the intestine. The increase in blood glucose that follows the ingestion of fructose is delayed because fructose has to go through gluconeogenesis in the liver to be modified into glucose (Pontremoli and Grazi 1968; Sestoft 1983). The length of the delay in the increase in blood glucose after a fructose meal depends on the levels of liver glycogen and is shorter with larger glycogen stores (Sestoft 1983, Novin et al. 1985). This lag may explain why fructose is preferred over sucrose in long term 3 h trials which permit glycogen accumulation but not in short term trials.

Cedar Waxwings are able to digest sucrose, although the efficiency with which they do so is less than that with which they digest glucose and fructose. Why do Cedar Waxwings absorb monosaccharides more efficiently than the disaccharide sucrose? The mean retention times I estimated for Cedar Waxwings are extremely short (grand mean of all the experiments = 40.73 min, see also Holthuijzen and Adkisson 1984), they are even shorter than those of Rufous Hummingbirds (Selasphorus rufus mean retention time ± SE = 45

± 3 min, Karasov et al. 1986). The weight of Rufous Hummingbirds is ca 3g (Karasov et al. 1986), and that of Cedar Waxwings is about 30 g. In contrast with Cedar Waxwings, hummingbirds show extremely high extraction efficiencies (ca 99%) when fed sugar solutions of both monosaccharides and sucrose (Hainsworth 1974, Karasov et al. 1986). It may be that the extremely fast passage rates of food in Cedar Waxwings impede the efficient digestion of a substrate such as sucrose which has to be hydrolyzed before it is absorbed. Researchers have historically assumed that high food passage rates are a typical trait of frugivorous birds (Wetmore 1914; McKey 1975; Moermond and Denslow 1985). This assumption has received surprisingly little empirical support, and data comparing passage rates of frugivorous birds with those of birds with other feeding habits are scant. Herrera's (1984) work on Mediterranean frugivores is one of the few good comparative examples. If high food passage rates are indeed typical of frugivores, and if fast passage rates hinder the digestion of sucrose relative to the digestion of hexoses, then I hypothesize that frugivorous birds are less efficient in digesting sucrose than in digesting glucose and fructose. I also hypothesize that a consequence of the constraint imposed by high food passage rates is that frugivorous birds prefer the monosaccharides glucose and fructose over sucrose.

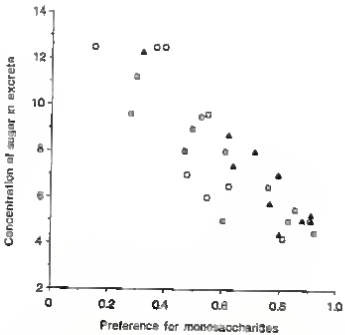

Fig 2-1 Relationship between preference for monosaccharides over sucrose and sugar concentration in excreta Data for three experiments in which glucose (circles, $r_s = -0.87$, $p < 0.01$), fructose (squares, $r_s = -0.60$, $p < 0.01$), and a mixture of glucose and fructose (triangles 3, $r_s = -0.78$, $p < 0.01$) were paired with sucrose Each point represents an individual bird in one experiment (the same 10 birds were used in each experiment) Concentration is expressed as weight per unit weight of sucrose equivalents

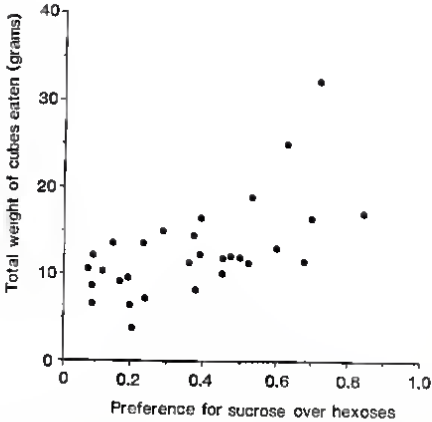

Fig 2-2 Relationship between total weight of cubes consumed and preference for sucrose over monosaccharides (r_S = 0 62, p < 0 001)

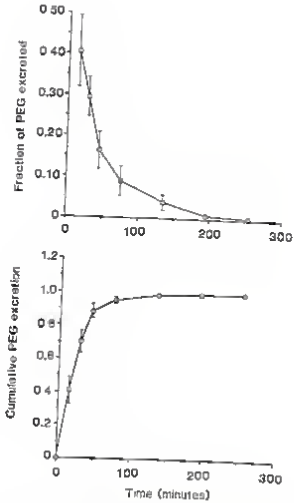

Fig 2-3. Excretion of the inert marker PEG as a function of time. Top: fraction of total excreted PEG excreted during the previous interval, bottom cumulative excretion. Each point is the weighted mean of three experiments, bars are standard errors. Six birds were used in each experiment

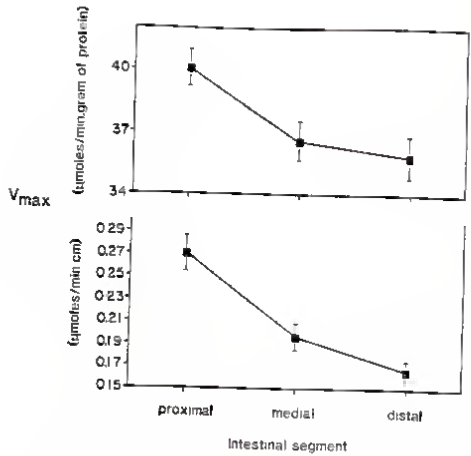

Fig. 2-4. Maximal sucrose hydrolysis (V_{max}) as a function of intestinal position in Cedar Waxwings. Bars are SE, hydrolysis is standardized to grams of protein in homogenate (top) and length of intestine (bottom). The value of V_{max} was estimated independently for each segment and each one of 3 birds using a non-linear least squares routine.

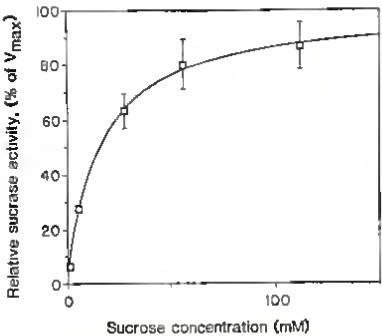

Fig 2-5. Sucrase activity in mucosal homogenates of Cedar Waxwings as a function of sucrose concentration. Vertical bars give SE. Relative sucrose hydrolysis was calculated from values of V_{max} that were independently estimated for each intestinal segment and each bird. The curve is a Michaelis-Menten equation calculated using a V_{max} equal to 100% and a K_m equal to 15.5 mM.

Table 2-1 — Series 1 Results of experiments pairing sugar cubes with pure sugar cubes. The same 10 birds were used in all experiments. Preference is equal to the proportion of sugar cubes consumed. Preference was calculated for each bird and the mean preference for the ten birds tested against the null hypothesis of no preference (i.e. mean preference = 0.5)

	Glucose	Fructose	Sucrose	Glucose+Fructose
Preference for sugars ± SD	0.80 ± 0.16*	0.76 ± 0.10*	0.80 ± 0.12*	0.81 ± 0.06*
Total eaten ± SD (grams)	11.5 ± 3.3	14.6 ± 5.4	23.6 ± 7.8	10.8 ± 3.6
Refractometer reading of excreta ± SD[a]	4.0 ± 0.7	4.5 ± 1.1	12.1 ± 2.6	[illegible]

* Mean significantly greater than 0.5, $p < 0.005$, N=10

[a] An estimate of percent sugar in excrete

Table 2-2 Series 1. Proportions of pure agar and sugar cubes dropped (± SD)

	Experiment			
	Glucose	Fructose	Sucrose	Glucose+Fructose
Proportion dropped (pure agar cubes)	0 85 ± 0 05	0_88 ± 0_01	0_71 ± 0 03	0 98 ± 0 03
Proportion dropped (cubes with sugar)	0 09 ± 0 06	0 06 ± 0 01	0 08 ± 0 08	0 12 ± 0 08
Number of birds observed	2	4	4	4

Table 2-3.- Preferences for sugars at left of matrix over sugars at top of matrix (Series 2) in 1 5 h trials. Preference is the proportion of sugar at top of matrix eaten, the same 10 birds were used in all experiments

	Glucose	Glucose+Fructose	Sucrose	Fructose
Glucose	---	0 54 ± 0 19 ns	0 73 ± 0 17*	0 72 ± 0 09*
Glucose+Fructose		---	0 67 ± 0 14*	0 70 ± 0 17*
Sucrose			---	0 55 ± 0 19 ns

* Mean significantly different from 0 5, $p < 0\ 01$, N=10

ns Mean not significantly different from 0 5, $p > 0\ 05$

Table 2-4 - Extraction efficiency of glucose, fructose and sucrose (N=6 birds/sugar, the same 6 birds were used in all experiments)

	Sugar		
	Glucose	Fructose	Sucrose
Extraction efficiency (%) ± SD	92 ± 1.5	88 ± 3.5	61 ± 1.2

CHAPTER 3
SUGAR PREFERENCES IN HUMMINGBIRDS: THE INFLUENCE OF SUBTLE CHEMICAL DIFFERENCES ON FOOD CHOICE

In the process of feeding on nectar, hummingbirds transfer pollen from one flower to another (Trelease 1881). Hummingbird preferences for plants producing different types of nectar can result in changes in the frequency of preferred nectar in plant populations (Baker and Hurd 1968). Ecologists have generally assumed that the diversity of rewards that plants offer is a reflection of pollinator choice (Baker and Hurd 1968, Howell 1974), but there are few data demonstrating such preferences (see Hainsworth and Wolf 1976 and Stiles 1976 for hummingbirds, and Waller 1972 for bees).

Nectar is a dilute solution of different sugars containing small amounts of aminoacids and electrolytes (Baker and Baker 1982a, Hiebert and Calder 1983, Pyke and Waser 1981). The sugars most commonly encountered in nectar are sucrose, fructose and glucose in varying proportions (Percival 1961). These sugars have approximately the same energetic content per unit gram (16.48X10^3 Joules/gram, CRC Handbook of Chemistry and Physics 1979), but vary in their chemical structure, and are digested, absorbed and metabolized along different pathways by vertebrates (Alpers 1987). The distribution of sugars in the nectars of plants pollinated by different groups of animals is a good example

of the diversity of rewards usually assumed to be molded by the preferences of pollinators. Baker and Baker (1983) have shown that plants pollinated by hummingbirds produce sucrose-dominant (sucrose/(glucose+fructose) > 0.95) or sucrose-rich (0.5 < sucrose/(glucose+fructose) < 0.95) nectars. Plants pollinated by "perching birds" (a broad category including passerines of many Old and New World families, Stiles 1981), in contrast, secrete hexose-dominant nectars almost exclusively (sucrose/(glucose+fructose) < 0.1). This pattern has not received an adequate evolutionary explanation. Here I examine the sugar preferences of hummingbirds to determine if these preferences can indeed explain the differences in sugar constituents among these two groups of plants.

The effects of variation in energetic content on food preferences have been explored in detail by many studies (see Stephens and Krebs 1986 and references therein). Effects of the chemical nature of different energy sources on feeding preferences have received considerably less attention, however (Speakman 1987). Nectar-feeding birds and the sugars they feed on are one of the simplest natural systems available to explore the effects of the chemical structure of food on feeding choices. Here I explore the effects of the subtle chemical differences among foods of equal energetic content (the sugars sucrose, glucose and fructose) on the preferences of hummingbirds, the most specialized group of nectar-feeding birds (Stiles 1981).

I will describe a series of experiments conducted to measure the preferences of three species of Mexican hummingbirds (Amazilia rutila, Cynanthus latirostris and Chlorostilbon canivetii) for sucrose, glucose, fructose and a 1:1 mixture of glucose and fructose. I will also explore whether these preferences can be altered by a change in the birds' diets. I will then suggest a set of hypothetical proximate physiological mechanisms to explain the preferences found, and describe experiments conducted to examine them. Because the hypotheses examined in the second section depend on the results of the first, I present the methods and results of the two sections separately.

Methods and Results

Bird Care and Housing

I captured eight to ten birds of each species with mist nets in the vicinity of the Estación de Biología Chamela UNAM, Jalisco, México. Birds were housed individually (50 X 50 X 50 cm cages) under ambient temperature and photoperiod (except during excretion measurements, see below) in a room illuminated with external light and two fluorescent lamps. Between experimental periods, birds were fed a 17.1% sucrose solution made with commercial table sugar and tap water. A vitamin supplement was added to the sucrose solution twice a week. Birds were provided with free-flying fruit flies at all times.

Sugar Preferences

Experimental rationale and methods

With few exceptions, hummingbirds feed predominantly on nectars dominated by sucrose (Stiles 1976, Baker and Baker 1983). Remarkably few plants produce nectars containing glucose or fructose alone (Baker and Baker 1983). Thus, I predicted that the preferences of hummingbirds for equicaloric solutions would be ranked in the following order: sucrose>glucose+fructose>single hexoses(glucose or fructose). To test this hypothesis I presented hummingbirds with all possible pairwise combinations of sucrose, a 1:1 mixture of glucose and fructose (henceforth called hexose mixture), glucose alone, and fructose alone.

Two sugar solutions (17.1%, wt/total volume) were presented simultaneously in glass tubes. The tubes (internal diameter = 7.6 mm) had a 42 cm vertical section and a lower 7 cm section that was bent upward at a 45° angle, the tip of the lower section was tapered into a hole (internal diameter = 2.5 mm) from which the birds drank. Both tubes were at the same distance (c.a. 30 cm.) from a single perch and 5 cm apart. The solutions were prepared with distilled water and reagent grade sugars. Trials lasted 4 h and were conducted from 0800 to 1200 and from 1400 to 1800. At the beginning of each trial the position of the tubes was randomized. At the end of each trial, consumption of solutions was measured to the nearest mm and then converted to volume units. Preference

for sugar A over sugar B, was calculated as the ratio of the consumption of sugar A divided by total consumption (A+B).

For each species and each pairwise comparison of sugars, I obtained an estimate of mean preference using 6 birds and 6 replicated trials per bird. The interindividual variance in preference (i.e. the variance among the 6 individual means) was used to construct a confidence interval for the mean preference of each species. I used this confidence interval to test the null hypothesis that preference was not significantly different from 0.5 (the indifference point where consumption of both sugars tested is equal) using one sample t-tests on arcsin√ transformed preference values.

Many hummingbirds feed on hexose-dominant nectars if these are available (Stiles 1976, Baker and Baker 1983, Gryj et al. 1990). Thus, in the field the sugar composition of the current diet may influence the preferences of hummingbirds. To examine whether shifting diets changed the preferences of hummingbirds, I fed 6 birds of each species a sucrose diet for 20 days and obtained an estimate of their preference for sucrose over a hexose-mixture (4 trials per bird). The same birds were then fed a hexose-mixture for 20 days and re-tested (4 trials per bird). I used one-tailed paired t-tests to detect increases in the preference for the hexose mixture.

Results

All three species exhibited the following ranking in their preferences:

sucrose > hexose mixture > solutions of single hexoses

(Table 3-1)

This ranking is the one predicted. In addition, all significantly preferred glucose to fructose solutions (Table 3-1). The preference for the hexose mixture did not increase significantly when the maintenance diet was changed from sucrose to a hexose mixture (Fig. 3-1). In fact, the preference of C. latirostris for the hexose mixture appeared to decrease after the diet change (Fig. 3-1).

Digestion of Sugars

Experimental rationale and Methods

Items of identical caloric content but different chemical composition can have different profitabilities if they are assimilated in the digestive system with differing efficiencies and/or if they are digested at different rates and hence vary in the digestive "handling time" required for assimilation (Speakman 1987, chapter 5). To determine if sugar preferences in hummingbirds were correlated with differences in digestive processing, I measured the efficiency with which hummingbirds assimilated sucrose, glucose and fructose, and compared the time required to process solutions of sucrose, glucose and hexose mixtures in the digestive system.

Assimilation efficiencies

I obtained estimates of digestive efficiencies for sucrose, glucose and fructose by measuring apparent assimilated mass coefficients (AMC*, Karasov 1990) in 24 h trials. AMC* was calculated as:

AMC* = (dry matter intake-dry matter excreted)/(dry matter intake).

Because endogenous metabolic wastes are mixed with feces in the cloaca of birds, AMC* underestimates true assimilated mass coefficients (Robbins 1983). AMC*, however, permits a comparison of assimilation efficiency among different sugars.

Birds were fed ad-libitum with sugar solutions (17 %) and kept in cages with plastic sheets lining the bottom. Each cage had a single small perch (10 cm of length). Plastic sheets were removed and excreta were collected at hourly intervals. Most of the excreta produced fell directly under the perch and was readily collected with microcapillary tubes. Losses due to excreta adhering to the plastic sheets were minimized by frequent collection (1 h). Birds were deprived of fruit flies 48 hours before each experiment, and to avoid contamination from the previous day's diet, birds were fasted overnight and for an hour after sunrise. Test solutions were offered one hour after sunrise, and excreta were collected at one hour intervals until sunset. Excreta produced during the night was collected the following morning. Birds were not fed for an additional hour the next day and all excreta produced in this interval were collected

Consumption of sugar solutions was measured at one hour intervals. Excreta were dried to constant weight at 60° C.

Processing time

Digestive efficiencies are not sufficient to predict the rate of energy intake from digestion, the time required to process the energy obtained is also needed (chapter 5). Because I found no differences in AMC* (see Results), I hypothesized that differences in processing rates could account for the preferences found. I predicted that the ranking in the time used to process sugar solutions in the gut would be the following:

pure hexoses > hexose mixture > sucrose.

Chemical differences among sugars and their possible physiological consequences provide the mechanisms that can account for this ranking.

The hexoses glucose, and fructose, are transported across the intestinal membrane by independent carrier systems (Sigrist-Nelson and Hopper 1974). Consequently, solutions of these single monosaccharides that are absorbed by a single system working by itself should be absorbed at a slower rate than aquicaloric solutions of mixtures that are absorbed by two independent carrier systems. It is assumed that most of the transport of monosaccharides in the hummingbird intestine is mediated by intestinal carriers (Karasov et al. 1986).

The osmotic pressure exerted in the intestinal lumen by a 1:1 mixture of glucose and fructose is twice as high as

that of an equicaloric solution of sucrose. If the rate of delivery of food from the crop and stomach to the intestine is mediated by the osmotic pressure in the intestinal lumen (Gibson et al. 1968, McHugh et al. 1982), then sucrose may be processed at a faster rate than an equicaloric mixture of hexoses. Sucrose is not absorbed in the intestine as such, but has to be first hydrolyzed into its monosaccharide components glucose and fructose (Alpers 1987). The osmotic hypothesis assumes that the limiting step in the absorption of monosaccharides from sucrose is absorption rather than hydrolysis. In vitro measurements of sucrase activity in hummingbirds support this assumption (chapter 4).

I compared processing times for sucrose, hexose mixture and glucose solutions at two concentrations (17.1% and 34.2%). As a processing time index (PTI) I used the reciprocal of the rate of excretion, because the time required to process one intestinal volume equals the ratio of intestinal volume/flow rate (Penry and Jumars 1987). If intestinal volume remains constant, the reciprocal of excretion rate (an index of intestinal flow rate) measures the time required to process each volume unit of digesta. The rationale and shortcomings of using PTI are discussed by Owen (1972, 1975) and Sibly (1981).

To obtain excretion rates, test solutions were presented at dawn to hummingbirds. After 2-3 hours of feeding, excreta were collected and measured in microcapillary tubes at five minute intervals during two hours. Excreta were collected by

removing the plastic sheets lining the bottom of the cages. Each 5 minute-interval measurement was used as a replicate. To examine differences among the mean PTIs of different sugars, I used a randomized block design with 24 replicates per bird (5 *C. latirostris* individuals and 4 *C. canivetii* individuals). Because I measured excretion rates in only two *A. rutila* individuals, I analyzed each individual separately using one way analysis of variance (24 replicates per sugar solution). In all excretion rate experiments, birds were housed at 21° C (± 2°C) and under a 12D-12N hrs light cycle.

Results

Digestive efficiencies

Hummingbirds digested all sugars with extremely high and very similar efficiencies. AME* for all sugars was ≥ 97% in all cases (Table 3-2) and not significantly different among sugars (paired t-tests, $p > 0.1$).

Processing times

In *C. latirostris* and *C. canivetii* processing time indices were significantly different among sugars at both concentrations (17.1% and 34.2%, Figs. 3-2a and 3-2b). The two *A. rutila* individuals tested also exhibited significant differences in processing time indices among sugars (Fig. 3-3). Contrary to my prediction, a priori t-contrasts indicated no significant differences among mean processing time indices of sucrose and the hexose mixture. Significant differences

between glucose and both sucrose and the hexose mixture were found in all but one experiment (Fig. 3-3b). As predicted, hummingbirds take longer to process glucose solutions than either hexose-mixtures and sucrose solutions.

Discussion

Sugar Preferences

The sugar preferences of hummingbirds have been examined in two previous studies (Stiles 1976, and Hainsworth and Wolf 1976) with conflicting results. Stiles (1976) examined sugar preferences in four species (*Calypte anna*, *Selasphorus rufus*, *Archilocus alexandri* and *Thalurania furcata*) and found that all of them significantly preferred sucrose over equicaloric solutions of single monosaccharides. When sucrose was paired with a mixture of glucose and fructose, sucrose was significantly preferred by two species (*C. anna* and *S. rufus*) but one species (*A. alexandri*) was indifferent. Thus, my results indicate strong preferences that agree with those presented in his (Stiles 1976). In contrast, Hainsworth and Wolf (1976) found no strong preferences, and argued that in hummingbirds sugar composition has little effect on food choice.

Differences in test choice protocols may explain the discrepancy between these studies. Stiles' (1986) and I exposed birds to test solutions in the same position for relatively long periods (12 and 4 hours respectively), whereas Hainsworth and Wolf (1976) alternated the position of

the feeders every 0.5 h. Because hummingbirds take a relatively small number of meals in 0.5 h (2.4 to 4.5, table VI in Wolf and Hainsworth 1977), this interval may be too short for sampling of both feeders and for the establishment of clear preferences.

Combining results from this study with those of Stiles, hummingbirds appear to show weak preferences among sugars in brief exposure tests, but strong preferences in long exposure tests. In long exposure tests the preference ranking of sugars in five species of hummingbirds is:

sucrose > mixture of hexoses > glucose > fructose

(Stiles 1976, this study). One species (*A. alexandri*) deviates from this ranking in that it appears to show no preference for sucrose over mixtures of glucose and fructose.

The preference that hummingbirds show for different sugars contrast with those exhibited by Cedar Waxwings (*Bombycilla cedrorum*) and European Starlings (*Sturnus vulgaris*). These two passerines strongly prefer mixtures of glucose and fructose over sucrose (Martinez del Rio and Stevens 1989, chapters 1 and 2). The variation in sugar preferences among bird species indicates that the sugars sucrose, glucose, and fructose, are not equivalent for different species of birds even though they are quite similar chemically.

Pre- and Post-ingestional Correlates of Sugar Preferences

The preingestion handling time of sugar solutions depends on nectar viscosity and surface tension (Pyke and Waser 1981, Kingsolver and Daniel 1983). In the range of concentrations used in these preference tests, the differences among sucrose, glucose and fructose solutions in viscosity and surface tension are extremely small and have little effect on the rate of intake (Heyneman 1983). Hence, pre-ingestion handling times are unlikely to generate differences in profitabilities that can account for the sugar preferences exhibited by hummingbirds.

I hypothesized that post-ingestional variation in the digestion and absorption of different sugars by hummingbirds could explain their preferences. I was unable, however, to find differences in the efficiency with which hummingbirds assimilated different sugars. Hummingbirds assimilated sucrose, glucose and fructose with the same and extremely high efficiency (see also Hainsworth 1974). I was also unable to find differences between the rate at which hummingbirds processed sucrose and 1:1 mixtures of glucose and fructose solutions in the digestive system. Apparently the preference of hummingbirds for sucrose over a mixture of hexoses cannot be explained by differences in processing rate in the digestive system.

Hummingbirds processed glucose solutions at significantly slower rates than solutions of sucrose and 1:1 mixtures of glucose and fructose. This result is consistent

with the existence of two independent transport systems for glucose and fructose in the small intestine (Sigrist-Nelson and Hopper 1974) Solutions of single monosaccharides (glucose or fructose) that are absorbed by a single system working by itself, should be absorbed at a slower rate than equicaloric solutions of 1 1 mixtures that are absorbed by two independant carrier systems working simultaneously. Sucrose and hexose mixture solutions probably have higher profitabilities for hummingbirds than solutions of single hexoses

Hummingbirds showed a very strong preferance for glucose over fructose (see also Stiles 1976) Cedar waxwings also preferred glucose over fructose (chapter 2), suggesting the hypothesis that this preference may be widespread among birds The metabolic responses of animals to glucose and fructose meals are very different and may explain the preference of birds for glucose over fructose Fructose meals can be followed by hypoglycemia (Roudybush 1970; Shafir 1985) and fructose does not cross the blood-brain barrier and therefore cannot be oxidized by the brain (Oldendorf 1971) Glucose meals, in contrast, cause post-prandial hyperglycemia (Hazelwood 1986) and glucose freely crosses the blood-brain barrier (Oldendorf 1971) Consequently, glucose may be more efficient than fructose at stimulating the cerebral chemoreceptors responsible for suppressing hunger (Stricker and Rowland 1978; Stricker et al 1977) Thus, the preferences of birds for simple sugars may be influenced by

physiological events that occurr after sugars are tasted, ingested and absorbed in the digestive system

Why do Hummingbirds Prefer Sucrose?

A digestive mechanism seems an unlikely explanation for the preference of hummingbirds for sucrose over mixtures of glucose and fructose Sucrose and 1:1 hexose mixture solutions appear to be equally profitable and to cause similar post-prandial physiological effects in hummingbirds. Then why do hummingbirds prefer sucrose? At the moment I cannot give a satisfactory answer to this question The ingestion of sucrose vs hexoses may have physiological consequences that are not reflected in food processing time. In addition, sugar choice may be influenced by behavioral/ecological processes that occurr in the field and that are absent in the simplified laboratory environment where I conducted the preference tests The previous feeding history of wild caught hummingbirds may influence their feeding choices in the laboratory Studies on the feeding preferences of a variety of vertebrates and invertebrates have indicated that foods experienced early in life are preferred over those experienced later (Bronstein et al 1975; Dethier and Goldrich 1971; Hess 1964) Hess (1964) has called this phenomenon "food imprinting". Perhaps nestling hummingbirds become "imprinted" to the sucrose-rich nectars regurgitated by their mothers (Dickey 1915, Bent 1964, Carpenter and Castronova 1980)

I attempted unsuccessfully to reverse the preferences of hummingbirds for sucrose by exposing them to the non-preferred diet, a hexose mixture. My results attest more to the strength of the preferences of hummingbirds than to their lability (see Fig. 1-3 and Stiles 1976).

The general pattern that hummingbirds feed on sucrose-rich flowers has exceptions. A few species (e.g. Heliomaster constantii and Amazilia violiceps in the tropical deciduous forests of Western Mexico) seem to feed predominantly on the hexose-dominant nectar of bat pollinated flowers (e.g. Agave spp., Pseudobombax spp., Ceiba spp., Baker and Baker 1983). The sugar preferences of these species are unknown.

Sugar Preferences in Hummingbirds and the Sugar Constituents of Nectar

Hummingbird pollinated plants produce nectars with a high proportion of sucrose, whereas passerine pollinated plants secrete nectars with a balanced mixture of glucose and fructose, and extremely low amounts of sucrose (Baker and Baker 1982a, 1983). Martínez del Río and Stevens (1989) hypothesized that the preferences of birds are the selective pressure responsible for maintaining the sugar composition in the rewards that plants offer. I have argued, however, that sucrose and hexose mixture solutions appear to be equally profitable for hummingbirds. Maybe plants play a more important role than it has been recognized.

Sucrose is the major form in which energy is translocated in most plant species (Hawker 1985), and it is probably cheaper for plants to secrete sucrose directly from the phloem than to hydrolyze it in the nectaries into glucose and fructose (Fray-Wyasling 1955). It is likely that in the absence of pollinator selection against sucrose, sucrose producing phenotypes are advantageous. Hummingbirds are perhaps the most specialized nectar-feeding birds (Stiles 1981), and their digestive systems are extremely well suited to digest and absorb a sucrose diet efficiently. Hummingbirds have the highest rates of carrier mediated intestinal glucose transport reported among vertebrates (Karasov et al 1986) and their rates of intestinal hydrolysis of sucrose are extremely high (chapter 4). These digestive traits allow them to use sucrose as efficiently as hexose mixtures. The digestive adaptations of hummingbirds allow hummingbird-pollinated plants to secrete sucrose dominated nectar. Passerine-pollinated plants, in contrast, are visited and pollinated by a diverse group of relatively unspecialized species (Gryj et al. 1990, Oatley and Skead 1972, Stiles 1981), some of which cannot use sucrose as efficiently as the readily assimilable monosaccharides glucose and fructose. In these plants the presence of sucrose in nectar can act as a floral filter that excludes valuable potential pollinators. The distribution of floral nectar sugar constituents in bird-pollinated plants appears to be the result of the

evolutionary response of plants to two sets of pollinators with contrasting degrees of digestive specialization

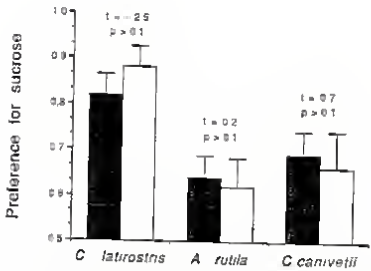

Fig. 3-1 - Effect of diet on sucrose preferences. Birds were fed a sucrose maintenance diet for 20 days and then offered a choice between sucrose and glucose+fructose solutions (Four 4 h replicates per bird, 6 birds). Birds were then changed to a glucose+fructose maintenance diet for 20 days and after this period, offered a choice between sucrose and glucose+fructose solutions (Four 4 h replicates per bird). The mean preference of four replicates per bird was used as an estimate of sucrose preference, and the change in mean preference was tested using one-tailed paired t-tests. Closed bars are grand mean preferences for the sucrose diet, and open bars are grand mean preferences for the glucose+fructose diet. Error bars are interindividual standard deviations. Note that the trend in C. latirostris is in the opposite direction to that expected

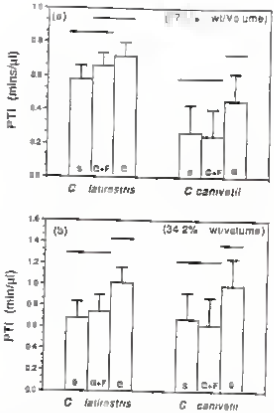

Fig 3-2.- Processing times (PTI) for different sugars at two different concentrations: 17 1% (a) and 34 2% (b). A randomized block design with 24 replicates per bird (5 *C. latirostris* and 4 *C. canivetii*) was used to examine differences among sugars. Bars are means and error bars are 95% confidence intervals. The lines above the bars join means that are not significantly different at the 0 05 level (a priori t-contrasts). S = sucrose, G+F = 1:1 glucose+fructose mixture, G = glucose

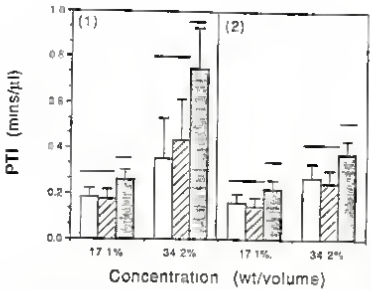

Fig. 3-3 - Processing times for different sugars in A. rufila. Only two individuals were tested and were analyzed separately. Bars are means (n = 24) and error bars are 95% confidence intervals. The lines above the bars join means that are not significantly different at the 0.05 level (a-priori t-contrasts). Sucrose = open bars, glucose+fructose = stipled bars, and glucose = solid bars.

Table 3-1 —Preferences for sugars in three species of hummingbirds. Sugars were presented in solution (17.1% wt/volume) in paired drinking tubes (4 h trials). Preference values for each combination of sugars are grand means (6 individuals and 6 trials per individual) ± interindividual standard deviations. S = sucrose, G = glucose, F = fructose and G+F = 1:1 glucose + fructose mixture.

Species	Comparison					
	S vs G+F	S vs G	S vs F	G+F vs G	G+F vs F	G vs F
Chlorostilbon canivetii	0.69 ± 0.05	0.88 ± 0.06	0.86 ± 0.05	0.72 ± 0.20	0.84 ± 0.10	0.89 ± 0.06
	t = 9.2[a]	t = 14.2	t = 15.2	t = 2.7	t = 7.6	t = 15.26
Cynanthus latirostris	0.79 ± 0.12	0.93 ± 0.06	0.88 ± 0.07	0.91 ± 0.06	0.79 ± 0.12	0.88 ± 0.05
	t = 4.4	t = 17.1	t = 13.3	t = 16.5	t = 6.1	t = 17.5
Amazilia rutila	0.63 ± 0.07	0.86 ± 0.09	0.83 ± 0.13	0.93 ± 0.06	0.78 ± 0.10	0.83 ± 0.08
	t = 4.5	t = 9.7	t = 6.7	t = 17.0	t = 7.[illegible]	[illegible]

[a] $t_{0.05,\,5} = 2.6$.

Table 3-2 - Apparent assimilated mass coefficients (AMC*) for sugars in three species of hummingbirds. Values are means ± SD

	AMC* ± SE			
	Sucrose	Glucose	Fructose	n
Species				
Amazilia rutila	0.97 ± 0.01	0.99 ± 0.01	0.97 ± 0.02	4
Cynanthus latirostris	0.99 ± 0.01	0.97 ± 0.02	0.98 ± 0.01	6
Chlorostilbon canivetii	0.98 ± 0.02	0.99 ± 0.01	0.97 ± 0.03	5

CHAPTER 4
DIETARY, PHYLOGENETIC AND ECOLOGICAL CORRELATES OF INTESTINAL SUCRASE AND MALTASE ACTIVITY IN BIRDS

Birds vary in the types and amounts of carbohydrates that they ingest To be assimilated, many carbohydrates have to be first hydrolyzed by pancreatic enzymes into disaccharides (Alpers 1987) Disaccharides are not absorbed as such by the intestine, and hence have to be hydrolyzed into their component monosaccharides by intestinal disaccharidases (Semenza and Corcelli 1986) The main theme I address here is the variation in the activity and affinity of two disaccharide-splitting enzymes among bird species sucrase-isomaltase and maltase Sucrase-isomaltase is a non-specific enzyme which hydrolyzes sucrose into glucose and fructose It also hydrolyzes maltose and isomaltose (the main products of starch digestion) into glucose Maltase hydrolyzes primarily maltose

I compare the intestinal enzyme activities of three species of hummingbirds, almost exclusive nectar-feeders, with those of eleven passarine species that span a wide range of dietary habits (table 4-1) I chose to use hummingbirds as the focus of this comparative study because they have high energetic demands that must be met by high rates of energy assimilation (Hainsworth 1981, Karasov et al 1986) Martinez del Rio and Stevens (1989) have suggested

that the sugar preferences of pollinators and seed dispersers may be correlated with the sugar composition of the nectars and fruit pulp that they consume, and that the preference between sucrose and the hexoses glucose and fructose may be mediated by intestinal sucrase activity. Hummingbirds are important pollinators of a variety of plants; therefore, their digestive physiology may influence, and be influenced by the chemical characteristics of the nectar that plants offer.

The specific questions I examine here are: Do different species of birds feeding on their natural diets vary in sucrase and maltase activity and in the affinity of sucrase for its substrate? Is there an allometric relationship between body mass and sucrose/maltose hydrolysis capacity as has been postulated by Karasov et al. (1985) for intestinal glucose uptake capacity and body weight for vertebrates? Since the sucrase-isomaltase complex has maltase activity (Norén et al. 1986), is there a correlation between sucrase and maltase activities? Is the relationship between maltase and sucrase different between hummingbirds and passerines?

Material and methods

Bird Capture and Care

Birds were captured with mist nets at the Estación de Biología Chamela (Jalisco, México, 19°30'N and 105°03' W). They were captured both in the dry deciduous forest that covers the hills and in the more humid subdeciduous forests

that border beds of dry streams and ravines. Hummingbirds were captured during August 1988 and kept in captivity for at least one month in 0.5 X 0.5 X 0.5 m cages under natural temperature and photoperiod. They were provided with 17.2% (wt/wt) sucrose solutions (commercial grade) and fruit flies ad libitum. Passerines were captured during October-November 1988. Relevant information on the natural history of each species included in this study is provided in table 4-1.

Sample Preparation and Enzyme Assays

Birds were anesthetized using metaphane and killed after the small intestine was excised. Passerines were killed within 2-3 h of capture. Small intestines were immediately chilled in ice-cold 1.02% saline and divided in three sections of equal length. Each tissue section was slit longitudinally, unfolded flat on a piece of paper and its outline was traced. The tissue was then blotted, weighed and stored in liquid N_2. An estimate of the nominal area of each section of intestine was obtained from the traced outline.

I assayed disaccharidase activities by colorimetry (Dahlqvist 1984). Prior to enzyme assays, tissues were thawed at 21°-23°C and homogenized (0.03-0.09 g tissue per ml 1.02% saline) for 30 sec with an Ultra Turrax homogenizer (mod. SDT 18.10, 2000 rpm). The reaction was started by adding 100 µl of tissue homogenate (diluted with 1.02% saline) to 100 µl of sucrose or maltose stock. Sugars (Contaminant-free sucrose and maltose, Sigma Chemical Co.) were dissolved in 0.1 M

maleate/hydroxyde buffer at a concentration and pH that varied depending on experimental conditions. After incubation at 40°C for 10 min, 3 ml stop/develop reagent stock was added. The stop/develop reagent was made by dissolving one bottle of "Glucose (Trinder) 315-500" reagent powder (Sigma Chemical Co., St. Louis Mo.) in 250 ml 1.0 M Tris/HCl (pH 7.0) plus 250 ml 0.5 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.0). Blanks were prepared by separately incubating 100 µl tissue homogenate at 40°C for 10 min, after which time 100 µl of disaccharide stock plus 3 ml of stop/develop reagent was added. The arrested reactions were then allowed to stand at 21°-23°C for 18 min, at which time the absorbances were measured at 505 nm using a Sequoia-Turner 330 digital spectrophotometer. Glucose standards (0-120 µg in 300 µl of 0.1 M sodium maleate buffer, pH 6.0) were also similarly reacted with the stop develop reagent. Based on absorbance measurements, sucrase and maltase activities were subsequently calculated as $\mu moles \cdot min^{-1}$ $(cm^2$ of nominal area$)^{-1}$, or $\mu moles \cdot min^{-1}$ (mg of wet tissue)$^{-1}$.

Standardization

I did not follow the traditional practice of standardizing intestinal disaccharidase activities by mg of mucosal protein (Dahlqvist 1968). Instead, I standardized intestinal disaccharidase activities by intestine nominal area (cm^2) and tissue wet weight (mg). This unorthodox standardization procedure was used because preliminary

experiments indicated that substantial activity was lost in the process of scraping the intestinal mucosa with a glass slide, this being the standard procedure for obtaining mucosa samples. Loss was especially noticeable in small birds (<30 g). Measuring enzyme activity from samples of scraped mucosa would have led to underestimation of total hydrolysis capacity. Nominal area and/or mg of wet tissue are commonly used to standardize intestinal glucose uptake rates (Karasov and Diamond 1983). Using the same parameters to standardize uptake and hydrolysis rates permits comparison of two digestive processes that are functionally and structurally related (Ugolev 1987, chapter 5). Karasov (1986) discusses the advantages and disadvantages of the use of different standardization procedures in the study of intestinal nutrient uptake.

Standardized enzyme activities of each intestinal section were obtained by dividing enzyme activity by the nominal area or wet mass of the section. The numerical sum of enzyme activities found in the three intestinal sections was used as an index of total hydrolytic capacity. A single number estimate of hydrolysis per unit nominal area (average enzyme activity) was used to compare enzyme activities across species. This variable was calculated by dividing summed enzyme activity by total nominal area or wet mass of intestine.

Determination of pH Optima, and Sucrase Kinetics

In the determination of pH optima I used tissue pooled from the three sections of the intestine, and a 0 05 M maleate/sodium hydroxyde buffer system with pHs ranging from 5 to 7 5. All subsequent measurements of maltase and sucrase activity were done at the optimal pH (to the nearest 0 5) of each species. Measurements of maltase and sucrase activity in the three different sections of the intestine were done at a fixed substrate concentration (0 028 M, Dahlqvist 1968). Kinetic parameters of sucrase were obtained from tissue pooled from the three sections of the intestine, and were measured at sucrose concentration varying from 0 005 M to 0 100 M

Data Analysis

Results are given as means ± SE (n = number of individuals or species). Maximal sucrase hydrolysis rates (V_{max}) and the apparent binding constants for sucrase (K_m*, the concentration of substrate at which the rate of hydrolysis equals $V_{max}/2$) were obtained using a nonlinear Gauss-Newton routine. Sucrase kinetic parameters were estimated separately for each individual. Standard least squares methods were used to estimate parameters of linear regressions. Correlation analysis was performed using product-moment and Spearman rank coefficients of correlation. I used product-moment coefficients of correlation as

indicators of goodness of fit of nonlinear functions but did not perform inferential statistics on them.

Results

Sucrase and maltase activities standardized by intestine nominal area (cm^2) and wet mass of tissue (mg) were tightly correlated with each other in a linear fashion both within ($0.96 < r < 0.98$ for all species, Fig. 4-1a) and among species ($r = 0.99$, $P < 0.001$, $n = 14$ spp, fig. 4-1b). In the results presented below I will standardize enzyme activities by intestine nominal area.

Sucrase and Maltase Dependence on pH

Sucrase pH optima were highly variable among species, ranging from less or equal than 5.0 in *Empidonax difficilis* to 6.5 in hummingbirds (Fig. 4-2). Species with low average sucrase activities tended to have lower sucrase pH optima than those with high average activities, as shown by the significant positive correlation between pH optima and V_{max} ($r_s = 0.86$, $P < 0.001$, $n = 12$ spp). Maltase pH optima showed little interspecific variability (ranging from 5.5 to 6). Maltase pH optima and average activity were not significantly correlated ($r_s = 0.138$, $P > 0.5$, $n = 14$ spp).

Sucrase and Maltase Activity

All species showed maltase activity but in widely varying amounts (Appendix 4-I, 4-II). Average sucrase

activity was highest among hummingbirds and barely detectable (average activity < 0 005 µmole/(min cm2)) in two species of muscicapids , Turdus rufopalliatus and Catharus aurantiirostris. Appendix 4-I) Average sucrase and maltase activities varied relatively little within species (coefficient of variation < 25%), but were very variable among species There was considerable variation in enzyme activity within restricted taxonomic groups such as families or subfamilies; Among Emberizidae and Tyrannidae average sucrase activity varied 8 and 4 5 fold, respectively (Appendix 4-1) The three hummingbird species, in contrast, showed no significant differences (ANOVA, $F_{2, 10} = 0.91$, $P > 0.5$).

Enzyme activity varied as a function of position along the intestine Both sucrase and maltase activity decreased distally in all species (figs 4-3a, 4-3b) Only sucrase is shown in these figures because maltase and sucrase activities were tightly correlated (see below)

Summed maltase and sucrase activities were positively correlated ($r_s = 0.68$, $P < 0.01$, $n = 14$ spp, Fig 4-4a) The correlation coefficient was substantially increased when only passerines were included in the analysis ($r_s = 0.90$, $P < 0.001$, $n = 13$ spp). The slope of the relationship between summed maltase and summed sucrase activities appears to be lower for hummingbirds than for passerines (because the sample size of hummingbird species is small, I refrained from using inferential statistics to examine this observation)

The difference between hummingbirds and passerines in the relationship of maltase and sucrase activities was accentuated when activity was standardized by intestinal nominal area (Fig 4-4b). Among passerines, average sucrase and maltase activities were highly and linearly correlated ($r = 0.95$, $n = 11$ spp., $P < 0.001$, fig 4-4b).

Hydrolytic Capacity and Body Mass

Summed sucrase activity and body mass were not significantly correlated ($r_s = 0.033$, $P > 0.5$, $n = 14$ spp). The correlation coefficient increased slightly when the two species with trace amounts of sucrase activity (T. rufopalliatus and C. auriptirostris) were deleted from the sample, but the correlation remained non- significant ($r_s = 0.22$, $P > 0.5$, $n = 12$ spp). Summed maltase activity was not significantly correlated with body mass when all species were included in the analysis ($r_s = 0.45$, $P > 0.1$, $n = 14$ spp). The correlation coefficient increased substantially, however, when T. rufopalliatus and C. auriantirostris were excluded from the analysis ($r_s = 0.71$, $P < 0.01$, $n = 12$ spp). The relationship between summed maltase activity and body mass appears to be better described by a power ($r = 0.75$, Fig 4-5) rather than a linear relationship ($r = 0.71$).

Sucrase Kinetics

In the range of concentrations studied (5-100 mM), sucrose hydrolysis exhibited saturable kinetics that were

adequately described by Michaelis-Menten functions (the coefficient of correlation for individual birds ranged from 0.95 to 0.99, Fig. 4-6). The value of K_m* spanned an order of magnitude, from 4.2 to 39.8 mM (Appendix I-4). The highest K_m* values (lowest apparent affinities) were found among hummingbirds, and the lowest in E. difficilis. Maximal sucrose hydrolysis rates (V_{max}) varied among species by two orders of magnitude (from 0.080 to 9.47 μmole/(min·cm^2 nominal area)). V_{max} and K_m* were positively correlated across species. The relationship between these variables was nonlinear and adequately described by a power function (r = 0.97, n = 14 spp, fig. 4-7).

Discussion

Interspecific Variation in Sucrase and Maltase Activity

Hummingbirds, among the smallest endothermic vertebrates, visit flowers using hovering flight and are exclusively diurnal (Wolf, Hainsworth and Gill 1975). These three characteristics impose relatively high energetic demands that can only be satisfied with high rates of energy intake and assimilation (Karasov et al. 1986). The intestinal rates of glucose carrier-mediated uptake of hummingbirds are the highest measured in vertebrates (Karasov et al. 1986). Hummingbirds feed on nectars that are extremely rich in sucrose (Baker and Baker 1983), and appear to prefer sucrose over hexose solutions (Stiles 1976, chapter 3). Sucrose hydrolysis, therefore, is an important component in the

digestion process of hummingbirds. Indeed, hummingbirds show maximal average rates of sucrose hydrolysis that are 2 to 110 times higher than those found in the 9 species of passerine birds having sucrase activity. The high sucrase activities of hummingbirds suggest a dietary component in the interspecific variation in sucrase activity.

Dietary habits appear also to be a determinant of sucrase activity within uniform taxonomical groups of passerines. Among tyrant flycatchers (Tyrannidae) E. difficilis is almost completely insectivorous and has lower average sucrase and maltase activity than the omnivorous Pitangus sulphuratus and Myiozetetes similis. Among emberizids, sucrase activity varies considerably, even within a single subfamily (Icterinae) or a single genus (Icterus). Because the feeding habits of the emberizids studied are quite generalized and/or poorly known, it is difficult to generalize trends in this taxon. It is noteworthy, however, that the highest average sucrase activity was found in I. spurius, a notorious nectar robber of plants pollinated by hummingbird and/or large bees (e.g., Malvaviscus spp., Tabebuia spp., and Erythrina crotaloides, Neil 1987, Morton 1979).

Two muscicapid species lacked significant sucrase activity. Lack of sucrase activity in birds has been reported for species belonging to three families that are closely related according to Sibley, Ahlquist and Monroe (1988, Muscicapidae, this report and Turdus migratorius, Karasov and

Levey 1990; Sturnidae, Sturnus vulgaris, Martinez del Rio and Stevens 1989; and possibly Mimidae, Dumetella carolinensis, Karasov and Levey 1990). Thus, the interspecific variation in intestinal sucrase activity in birds seems to have a phylogenetic component in addition to the dietary component discussed above. Only further comparative work will reveal the relative contribution of feeding habits and phylogenetic constraints to the variation of sucrose activity among birds. The activity of intestinal disaccharidases in mammals appears to be broadly correlated with feeding habits (Yonk and Western 1984), even among populations of a single species (see Kretchmer 1981 for a review of examples among populations of Homo sapiens).

With the exception of captive hummingbirds, the birds used in this study were feeding on natural (and largely unknown) diets before enzyme measurements. Hence, it is difficult to assess if the differences found reflect interspecific genotypic variation or simply dietary acclimation to differing diets in the period prior to capture. In chapter 4 I demonstrated large differences in sucrose activity among three species of birds maintained on identical diets. The variation in sucrose activity, therefore, is likely to have a genotypic component. Many species, including many mammals and two species of birds (Meleagris gallopavo and Gallus gallus), exhibit clear acclimation to dietary carbohydrate levels, however (Seal, Koldovsky and Reid 1988). Research with captive bird species

fed diets of known nutrient composition can help to elucidate the interaction between dietary acclimation and genotypic determination of intestinal disaccharidase activity (see Rosenzweig and Herman 1969; Buddington 1987)

The tight linear correlation between maltase and sucrase activity in passerines is probably a result of maltase activity in the sucrase-isomaltase enzyme complex (Sjöstrom et al. 1980). In passerines, as in many other vertebrates, the maltase activity of this enzyme complex constitutes an important portion of total intestinal maltose hydrolysis (Norén et al. 1986). In consequence, birds with very low sucrase activities seem to be relatively poor hydrolyzers of maltose.

Some of the bird species reported as poor sucrose digestors include considerable quantities of fruit in their diets (e.g. S. vulgaris, T. migratorius, T. rufopalliatus and D. carolinensis, Martin, Zim and Nelson 1951). Thus, the ability to digest sucrose and complex carbohydrates efficiently is not an essential requirement for avian frugivores (chapter 2, Martinez del Rio and Stevens 1989).

Hummingbirds and passerines exhibit clear differences in the relationship between maltase and sucrase activities (Fig. 4-4). To examine these differences, it is useful to consider that average maltase and sucrase activities are related by a linear function in passerines (Fig. 4-4b). The intercept of this function is significantly different from 0 ($t = 2.35$, $p < 0.05$) and is an estimator of average maltase activity in

the absence of sucrase activity. The value of the intercept represents the intestinal levels of a maltase that is independent of the sucrase-isomaltase complex (Sørensen et al. 1982). The slope of the relationship between maltase and sucrase activities is an estimator of maltase activity per standardized unit of the sucrase-isomaltase complex. Because the slope of the maltase-sucrase relationship is lower in hummingbirds, this interpretation suggests that the sucrase-isomaltase complex of hummingbirds has less maltase activity than that of passerines. Without more comparative data, it is difficult to determine if this difference between hummingbirds and passerine birds results from evolutionary adaptation to dietary differences or to phylogenetic affinities.

A linear relationship between maltase and sucrase activities is not exclusive of passerine birds. Reanalysis of previously published data sets suggests that the relationship holds inter- (12 species, Welsh and Walker 1973) and intraspecifically for primates (*H. sapiens*, Rosensweig and Herman 1969) and non-passerine birds (*M. gallopavo*, Seil et al. 1988). The slope of the relationship between maltase and sucrase activities varies considerably among groups (table 2-4). This variation suggests that among vertebrate species the sucrase-isomaltase complex varies in its maltase activity.

Several authors have used constancy in the ratios of intestinal disaccharidases as evidence for or against functional relationships among different molecular forms with

disaccharidase activity (Rubino, Zimbalatti and Aurichio 1964; Welsh and Walker 1973; Sell et al. 1988). The existence of a positive intercept in the relationship between the activities of two "functional" enzymes precludes the use of activity ratios for comparative purposes (see Packard and Boardman (1987) for a cautionary note on the use of ratios in comparative studies).

Hydrolytic Capacity and Body Mass

Summed sucrose and maltose hydrolysis rates show no or relatively weak allometric trends with body mass. Summed hydrolysis rates result from the integration of intestinal nominal surface area and area-specific rates of hydrolysis. The lack of a clear relationship between body mass and hydrolysis rates, therefore, can result from variation in nominal intestinal surface area among birds of similar masses or from variation in area-specific rates of hydrolysis of birds with similar body masses and intestinal surface areas. Among the 14 species of birds included in this study there is a very tight power relationship between body mass and nominal intestinal area (cm^2 nominal area = 0.66 (grams)$^{0.81}$, $r = 0.99$). Hence, the lack of significant allometric trends in intestinal hydrolytic activity appears to be a result of high interspecific heterogeneity in hydrolysis rates per unit of nominal area (see Fig. 4-4b).

My failure to find a relationship between summed disaccharidase activities and body weight is probably a

consequence of the use of a small sample of ecologically and taxonomically heterogeneous species. The analysis of allometric trends in intestinal hydrolysis rates may well yield interesting results if a larger and/or more homogeneous sample of species (e.g. hummingbirds) is used.

Unstirred Layer Effects and Variation in k_m and pH Optima

The range of interspecific variation of the Michaelis-constant of sucrase spanned almost an order of magnitude. Many studies have attributed physiological and evolutionary significance to variation in the affinities of enzymes (Farsht 1985). I suggest, however, that the interspecific variation in the apparent affinity of avian sucrase does not have evolutionary significance and can be explained by biophysical constraints on the techniques I used. Sucrase is anchored to the brush-border membrane of intestinal enterocytes (Hunziker et al. 1986), and I studied sucrase activities in whole tissue homogenates. In these preparations, sucrase is present in particle-bound form (Dahlqvist 1968), and hence separated from a well-mixed bulk sucrose solution by an unstirred layer. As hydrolysis proceeds sucrose becomes depleted in this layer adjacent to the membrane, and therefore, hydrolysis rates are measured at a solute concentration lower than bulk experimental concentration. This depletion should increase with the density of enzyme "copies" in the membrane (i.e. with V_{max}, the maximal rate of hydrolysis), so that high V_{max} values

should be associated with high K_m^* values (Fig 7-4). The estimation of V_{max} values appears not to be affected by unstirred layer effects (Laidler and Bunting 1980)

The relationship between K_m^* and V_{max} for enzymes bound to the surface of disks or spheres has been approximated by Sundaram, Tweedale and Laidler (1970) as

$$K_m^* = (K_m')^{1/2}(V_{max})^{1/2}(L)(D)^{-1/2}$$

In this equation K_m' is the "true" Michaelis constant of the enzyme in solution, L is the thickness of the unstirred-layer, and D is the diffusion coefficient of the substrate The results presented in figure 5 are consistent with the results of the theory of immobilized enzyme systems; K_m^* and V_{max} appear to be related by a power function with an exponent that is not significantly different from 1/2 (exponent = 0 45 ± 0 033, t = 1 51, P > 0 1) Karasov et al (1985) suggested that unstirred layer effects were responsible for interspecific variation in the apparent affinity of the intestinal glucose carrier and reported a relationship between K_m^* and V_{max} that is analogous to the one reported here for sucrase

Unstirred layer effects may also explain the observed upward shift in sucrase pH optima associated with increased activity Shifts in pH profiles resulting from increased concentration of enzyme can result if the enzymatic reaction releases or consumes acid or base in the region adjacent to

the membrane (Laidler and Bunting 1980). The mechanism of reaction of sucrase is poorly known, but it is believed to involve the protonation of the glycosyl oxygen of sucrose, the formation of an intermediate oxocarbonium ion and its stabilization by an OH^- into alpha-glucose (Norén et al 1986). The functioning of such a mechanism can influence the partitioning of hydrogen ions between the bulk solution and the immediate surroundings of the supporting membrane. Ngo and Laidler (1978) have reported changes in the pH profiles of enzymes entrapped in artificial membranes.

The function of intestinal enzymes in vivo is probably quite different from the function of intestinal enzymes as measured in vitro. In the whole live animal intestinal enzymes function while affixed to the interior of a tube, the intestine, whose walls have a complicated geometry and are covered by a glycocalyx (Ugolev and Tsvetkova 1986). The influence of unstirred layers on the apparent affinity of enzymes in vivo probably depends on the rate and pattern of flow of digesta through the intestine (Christensen 1984) and on the physical properties of the glycocalyx (Ugolev and Tsvetkova 1986). Unstirred layer effects limit the usefulness of comparisons between intestinal enzyme properties measured using different preparations such as brush-border membrane vesicles (Martinez del Rio and Stevens 1985), purified enzymes (Taravel et al 1983) and tissue homogenates (Sell et al 1988). The measurement of intestinal enzyme function in vitro is useful for comparative purposes (Wacker et al. 1983;

Vonk and Western 1984, this study) and for elucidating enzyme structure and mechanism of action (Hunziker et al 1986), but it does not replace the study of digestive processes in the intact animal (Ugolev 1987).

Ecological and Behavioral Consequences of Interspecific Variation in Disaccharidase Activities

Hummingbird-pollinated plants produce sucrose-rich nectars. Plants pollinated by passerine nectarivores, in contrast, secrete nectars containing mainly mixtures of the hexoses glucose and fructose (Baker and Baker 1983). The small bird-dispersed fruits that are typically consumed by passerine frugivores also contain mainly hexoses (Baker and Baker 1986, pers. com.). Perhaps the preferences of pollinators, seed dispersers, and seed and nectar parasites (birds that digest the seeds contained in fruits and/or consume nectar without contacting the reproductive parts of flowers) for different sugars act as selective pressures on plants. This hypothesis suggests two questions: Do sugar preferences vary among birds? and, What are the physiological traits that underlie these preferences? I examine these two questions below.

Intestinal sucrase activity can have a strong influence on sugar preferences, and presumably on the dietary choices of birds in the field (Martinez del Rio and Stevens 1989). European starlings (S. vulgaris), for example, lack sucrase activity and strongly prefer solutions of glucose and

fructose over solutions of sucrose (chapter 1). The inability to digest sucrose, resulting from the lack of physiologically significant sucrase activity, has been reported in five species of birds in three closely related families (see above). All of these species include fruit in their diets, and presumably act as important seed dispersers of many plant species (Johnson et al. 1985). Maybe the lack of sucrase in the muscicapid-sturnid lineage (sensu Sibley et al. 1988) is a good example of a phylogenetic constraint on the feeding behavior of birds that has profound ecological consequences. The extremely fast passage rates of some frugivores may preclude the efficient hydrolysis and absorption of sucrose relative to the hexoses glucose and fructose, even when these animals have significant amounts of intestinal sucrase (e.g. Bombycilla cedrorum, Chapter 2). Lack of sucrase activity is a sufficient but not a necessary condition for birds to prefer hexoses over sucrose.

Hummingbirds show very high sucrase activities and many species appear to prefer sucrose over hexose solutions (Stiles 1976; chapter 3). Crane (1975) postulated the existence of a glucose transport system independent of the commonly recognized sodium-dependent glucose carrier, and associated with disaccharidases (Malathi et al. 1973). In animals with high sucrase activities such as hummingbirds, this system could increase the rate of uptake of sucrose-derived hexoses above the rate obtained from free monosaccharides. The disaccharidase-dependent transport

system, however, lacks a satisfactory mechanistic explanation (Semenza and Corcelli 1986), and its existence has been questioned (Alvarado, Lharminier and Phan 1984). The preferance of hummingbirds for sucrose over hexosee remains a puzzling observation begging for a mechanism (chapter 3).

Birds show variation in their preferences for simple sugars, and these preferences seem to coincide with the kinds of sugars that they eat in the wild. Furthermore, the presence or absence of intestinal sucrose activity seems to account for the preference of some species of birds for hexoses over sucrose (Martinez del Rio and Stevens 1989). Some of these species are important seed dispersers and may have acted as a selective pressure on plants. The sugar preferences of birds, however, cannot be explained by a single, simple physiological mechanism, such as the presence or absence of intestinal sucrase. Sugar preferences are probably determined by relative rates of energy assimilation, and these are the result of the interplay between sucrose hydrolysis, glucose and fructose uptake, and intestinal retention times. chapter 5 integrates these three factors in a synthetic mathematical model of sugar digestion. Tests for this model and a complete picture of the sucrose-hexoses preference system in birds awaits the evaluation of these parameters in species of contrasting ecological habits and taxonomical affinities.

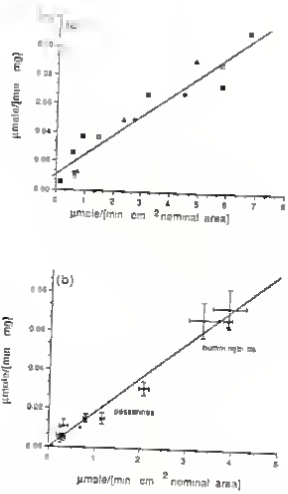

Fig. 4-1 Relationship between sucrase activity standardized by intestinal nominal area and tissue wet mass for a single species, Cynathus latirostris (a), and all species (b) In (a) different symbols are different individuals, different points are measurements taken at different regions of the intestine, the regression line was fitted only for descriptive purposes. Points in (b) are mean average activities for each species, bars are SE and the regression line equals Y = 0.018X (r = 0.99, P < 0.001).

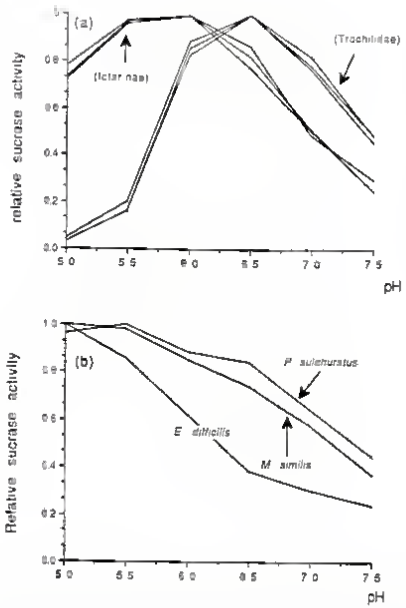

Fig. 4-2 Sucrase pH profiles. Activity was measured at 28 mM. Interindividual variation was small and is not shown. Sample sizes are the same as those of Appendix 1.

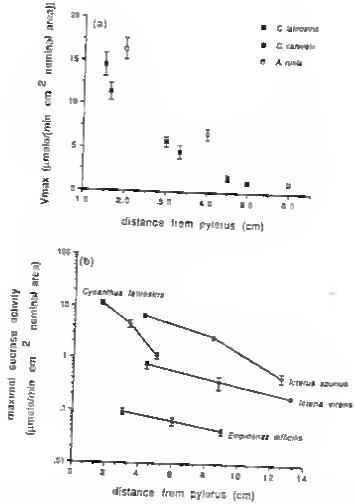

Fig. 4-3. Maximal sucrose hydrolysis as a function of intestinal position in three species of hummingbirds (a) and four species of birds (b). Vertical bars are SE. Hydrolysis is standardized by nominal area of intestine. Distances from the pylorus are average distances to the extreme distal end of the intestine section. Note that in all cases sucrase activity decreases distally along the intestine.

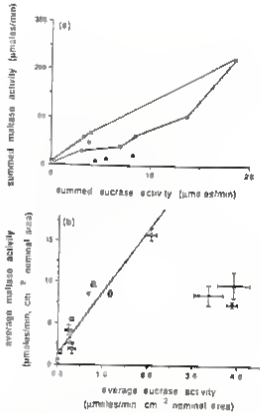
(a)
summed maltase activity (μmoles/min)
summed sucrase activity (μmoles/min)
(b)
average maltase activity (μmoles/min, cm⁻² nominal area)
average sucrase activity (μmoles/min cm⁻² nominal area)

Fig. 4-4. Comparison between maltase and sucrase activities. (a) Compares summed maltase with summed sucrase activity, and (b) compares activities standardized by intestinal nominal area. Open circles are passerines, and closed circles are hummingbirds. The polygon in (a), groups passerine species and is shown for descriptive purposes and as a homage to B. K. MacNab and his species. For passerines the estimated relationship between sucrase and maltase average activities equals Y = 1.39 + 7.38X ($r = 0.95$, $P < 0.01$). Values are means, and bars in (b) are SE. In both (a) and (b) enzyme activities were measured at 28 mM of substrate concentration.

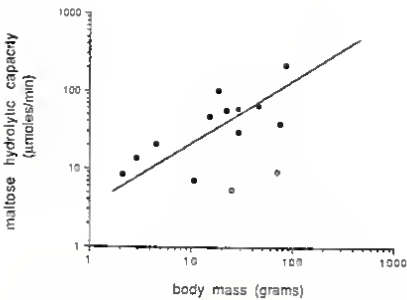

Fig. 4-5. Maltose hydrolysis capacity (measured at 28 mM) and body mass. Closed circles are species that had significant sucrase activity (> 0.005 µmoles/(min·cm^2)). Open circles are Turdus rufopalliatus and Catharus aurantiirostris. The parameters of the power relationship ($Y = 5.96X^{0.65}$) shown in the figure were estimated after removing T. rufopalliatus and C. aurantiirostris from the sample.

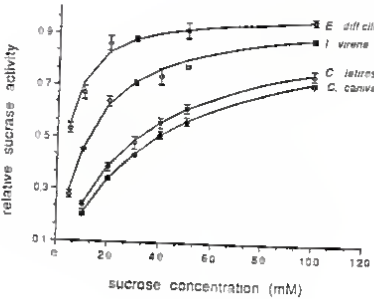

Fig 4-6: Sucrose hydrolysis as a function of incubation sucrose concentration for four species. Vertical bars are SE, sample sizes are those shown in Appendix II. Curves were fitted with a non-linear least-squares routine. Hydrolysis values are normalized to V_{max}.

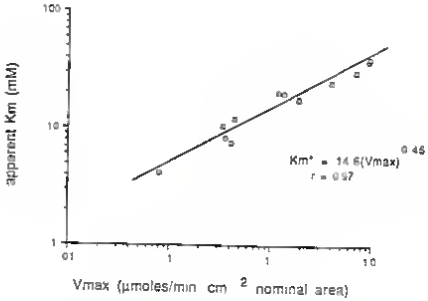

Fig 4-7. Apparent affinity as a function of maximal average sucrase activity. Points are mean species values. The power relationship between K_m* and V_{max} suggests that the measurement of K_m* is influenced by unstirred layer effects.

Table 4-1 - Natural history and dietary habits of three species of hummingbirds and eleven species of passerine birds of Western México. Numbers after comments refer to sources listed below

Family Species	Natural history and dietary habits
Trochilidae	
Chlorostilbon canivetii	Resident but extremely mobile, presumably tracking flower abundances, visits flowers as a trapliner (1, 2), often feeds on insect-pollinated flowers (1).
Cynanthus latirostris	Resident (1). Frequently visits flowers as a trapliner, may defend flower patches as territories (1, 3).
Amazilia rutila	Resident and the most common hummingbird species in the area (1). Very aggressive, often defends flower clumps as territories (4).

Tyrannidae	
Empidonax difficilis	Migrant. Mainly insectivorous in the nesting grounds (8) In the wintering area eats small amounts of fruit (9).
Pitangus sulphuratus	Resident. The most omnivorous flycatcher (5) Diet includes insects, small vertebrates and fruit (5, 6).
Myiozetetes similis	Resident. Insectivorous and frugivorous (6, 7)
Muscicapidae	
Catharus aurantiirostris	Resident. Mainly insectivorous, eats fruit (10).
Turdus rufopalliatus	Resident but highly mobile following fruit abundance. Consumes insects but is mainly frugivorous (10)

Emberizidae

Icteria virens	Migrant, in the nesting grounds consumes insects and fruit in about equal amounts (11, 12). In the wintering area eats insects, fruit and nectar (13).
Saltator coerulescens	Resident. Eats insects, fruit and flower petals and buds (10, 14).
Paroaria lecluncheri	Resident, mainly granivorous but also eats insects and nectar (10,13).
Icterus spurius	Migrant, in the nesting grounds consumes mainly insects and small amounts of fruit (15). In the wintering areas eats insects and nectar. Pollinator of some plant species and nectar robber of hummingbird-pollinated flowers (16, 17).

Icterus pustulatus	Resident	Eats insects, fruit and nectar (13, 14, 18, 19)
Cacicus melanicterus	Resident	Eats insects, fruit and nectar (8, 13, 14, 20)

1 Arizmendi and Ornelas 1990, 2 Fainsingar and Colwell 1978, 3 Das Granges and Grant 1977, 4 Schemske 1980 5 Fitzpatrick (1982), 6 Traylor and Fitzpatrick (1982), 7 Skutch (1980), 8 Sant 1942, 9 H Barlanga, unpubl data, 10 Martinaz dal Rio unpubl data, 11 Sant 1963, 12 Ehrlich, et al 1988, 13 Gryj, Martinaz del Rio and Bakar 1990, 14 Eguiarte and Martinaz del Rio 1985, 15 Martin at al 1951, 16 Morton 1979, 17 Naill 1987, 18 Crudan and Toledo 1977, 19 Eguiarta, at al 1987, 20 Bullock et al 1987

Table 4-2. Slopes of regressions between sucrase and maltase activities in selected groups. Because sucrase and maltase activity are measured in the same units slopes are dimensionless. Data were reanalyzed from original sources.

	Slope ± SE	r	n	source
Mammalia				
Primates	2.35 ± .48	.83	12 spp.	Welsh and Walker (1973)
Homo sapiens	3.20 ± .23	.97	13	Rosensweig and Herman (1969)
Aves				
Passeriformes	7.38 ± .82	.95	11 spp.	this study
Meleagris gallopavo	9.37 ± 2.49	.69	36	Sell et al. (1988)

CHAPTER 5
DIGESTION STRATEGIES IN NECTAR- AND FRUIT-EATING BIRDS AND THE SUGAR COMPOSITION OF PLANT REWARDS

The digestive processes and strategies of nectar- and fruit-eating animals, have been largely neglected (some exceptions are Walsberg 1975, Sorensen 1984, and Karasov et al. 1985). In this chapter I will use a mixture of simple mathematical and graphical methods to model the digestive processes of birds feeding on fruit and/or nectar. Although the models were constructed with birds in mind, the results probably apply to nectarivorous and frugivorous bats as well. The two areas I will address are how digestive constraints influence the preferance of birds for different sugars, and how digestive traits and strategies are modified by the concentration and sugar composition of food.

Because energy in nectar and the pulp of many fruits is mainly in the form of simple sugers (Baker and Baker 1983, Baker and Baker unpubl. data), the digestive processes of nectarivorous and frugivorous animals may be easier to study than those of animals feeding on more complex foods. In addition to sugars, many fruits contain relatively large amounts of fats and proteins (e.g. many Lauraceae, Burseraceae, and Palmae, Snow 1981b) and some birds feed almost exclusively on these fruits (e.g. Steatornis capensis, Snow 1962). I will restrict my discussion of the digestive

strategies of frugivorous birds to those species that feed on fruits containing sugars primarily.

Some birds show variation in preference among sugars (chapters 1, 2, and 3). These preferences are surprising because the sugars present in fruit and nectar (sucrose, glucose and fructose, Percival 1961) have nearly the same caloric content per unit gram. In chapter 4 I argued that sugar preferences are probably determined by relative rates of energy assimilation, and that these are the result of the interplay between sucrose hydrolysis, glucose and fructose uptake, and intestinal retention times. Here I integrate these three factors in a synthetic mathematical model of sugar digestion.

The sugar preferences of birds are not explained by standard foraging theory because, traditionally, the models of food choice have neglected the importance of digestive processes (Speakman 1987). By emphasizing digestion I hope to expand the models of food choice, and to provide a tool that may help understanding why animals vary in their preference for food items of the same energetic value but different chemical composition. In the construction of the models presented here I ignored the effect of pre-ingestional handling of food on the rate of energy intake. My models are not inclusive but rather serve to draw attention to a previously neglected component of foraging behavior.

Penry and Jumars (1986) championed the use of methods borrowed from chemical engineering to analyze digestive

strategies in animals. The approach suggested by Penry and Jumars (1986) consists of identifying the digestive tract of animals with a particular class of chemical reactors, and then proposing a set of chemical reactions occurring in the reactor (in this case the hydrolysis of sucrose and absorption of hexoses). The structure of the reactor and the nature of the chemical reactions determine a particular energy yield function that can be optimized to find combinations of chemical and physical parameters that maximize energy yield, given the constraints imposed by the design of the reactor and the characteristics of the "feed"

The Model

With few exceptions nectarivorous and frugivorous birds possess relatively simple guts consisting of a storage organ (a crop or a distensible esophagus) that presumably plays a negligible role in enzymatic digestion or absorption of sugars (Jung and Pierre 1933), a simple saccular stomach used to macerate and initiate the digestion of animal food and perhaps to separate pulp from seeds in frugivores (Walsberg 1975, Levey and Grajal unpubl.), and a tubular intestine in which hydrolysis and absorption of carbohydrates occurs (Forbes 1880, Wetmore 1914, McLelland 1979, Pulliainen et al. 1981, Duke 1986).

Penry and Jumars (1986, 1987) suggested that guts can be modelled as chemical reactors, and have identified the types of ideal chemical reactors analogous to digestive

organs. Sacular organs such as a crop or a stomach are probably analogous to continuous flow stirred tank reactors (CSTR) if the animal feeds continuously, or as batch reactors (BSR) if each meal is processed separately in discrete intervals (Penry and Jumars 1987). Tubular organs such as the intestine probably have little longitudinal mixing and are analogous to plug-flow reactors (PFR, Penry and Jumars 1987, see Levenspiel 1972 and Penry and Jumars 1987 for descriptions and nomenclature of ideal reactors). A gut such as that of fruit and nectar eating birds can be modelled as two reactors in series. First there is a continuous flow stirred tank reactor (the crop+the stomach). Coupled to it is a plug flow reactor (the tubular intestine). Because all the digestion of sugars takes place in the intestine, it may be reasonable to model the kinetics of the intestinal digestive processes of fruit and nectar eating animals as those of a PFR.

Kinetics of Sugar Absorption

In a PFR-like intestine the digestion of a single monosaccharide such as glucose (G) can be modeled as the rate at which the monosaccharide is absorbed (r_g); thus

$$dG/d\tau = - r_g \qquad (1)$$

The concentration of monosaccharide (G) is in μmoles/(μl of digesta), and τ is a time constant equal to the ratio of the

volume of the intestine to the rate of entrance of food to the intestine (v_0) and has units of time (minutes, see Levenspiel 1972). In ideal PFR-s, τ is equal to the mean residence time of the reactor (also called throughput time, Levenspiel 1972, Smith, 1987, notation follows Peary and Jumars 1986, 1987; all symbols are defined in Table 1).

At low to moderate hexose concentrations most monosaccharide absorption across the intestinal walls occurs through a carrier mediated process (facilitated diffusion for fructose (F), active transport for glucose, Alpers 1987, Karasov and Diamond 1983). Carrier mediated processes can be assumed to have Michaelis-Menten kinetics with a maximal rate of absorption (V_g) at saturating substrate concentrations and a Michaelis constant (k_g) reciprocally related to the affinity of the carrier system for the monosaccharide. In addition to using carrier mediated processes, vertebrates rely on passive diffusion to transport hexoses across the intestinal walls (Karasov et al 1985). If the concentration of monosaccharides in the blood stream remains constant, the passive component of monosaccharide transport is proportional to monosaccharide concentration in the intestinal lumen (Alpers 1987). The constant of proportionality in this relationship is called the passive permeability coefficient (k_d). The rate of intestinal absorption of a single hexose such as glucose can thus be modeled as

$$r_g = GV_g/(k_g + G) + k_dG \qquad (2).$$

The units of V_s, k_s and k_d are $\mu moles \cdot \mu l^{-1}\ min^{-1}$, $\mu moles\ \mu l^{-1}$ and min^{-1} respectively.

Unlike monosaccharides that are transported intact across the intestinal wall, sucrose (S) is not transported directly but has first to be hydrolyzed into its monosaccharide components, glucose and fructose. The membrane-bound intestinal enzyme sucrase is responsible for sucrose hydrolysis (Semenza 1976). The kinetics of uptake of monosaccharides (G,F) when sucrose is the feed of the chemical reactor, can therefore be modelled using the following system of differential equations:

$$\begin{aligned} dS/d\tau &= -r_s \\ dG/d\tau &= r_s - r_g \\ dF/d\tau &= r_s - r_f \qquad (3) \\ dA/d\tau &= r_g + r_f, \end{aligned}$$

where A is the total absorption of hexoses, and r_s is the rate of sucrose hydrolysis. Like most enzyme mediated reactions, the rate of sucrose hydrolysis by sucrase follows Michaelis-Menten kinetics.

To simplify eq. (3) I will collapse the monosaccharides glucose and fructose into a single category (H). This is a necessary step because the kinetics of intestinal uptake of glucose are well known, whereas those of fructose are

relatively unknown (Alpers 1987). The system describing the kinetics of uptake is

$$dS/d\tau = - r_s$$
$$dH/d\tau = 2r_s - r_h \qquad (4)$$
$$dA/d\tau = r_h$$

The maximal rate of hexose uptake (V_h) implicit in eq. (4) is equal to the sum of the maximal uptakes of glucose and fructose.

To model the uptake of monosaccharides from sucrose in the intestine as a system of ordinary differential equations in τ requires that the system be homogeneous; namely, that both substrates and enzymes are in solution. This assumption is clearly violated by all vertebrate intestines; both the enzyme that hydrolyzes sucrose and the protein carriers of monosaccharides are bound to the brush border membrane of the intestinal enterocytes. The system is therefore heterogeneous (liquid to solid). Biochemical engineers have explored the kinetics of heterogeneous systems, and a wealth of information exists on their behavior (Goldman and Katchalski 1971, Horvath and Solomon 1972, Koyasahi and Laidler 1974, chapter 4). The qualitative features of the heterogeneous system, however, are captured by the much simpler and tractable homogeneous system described by eq. (4).

An analytical solution does not exist for eq. (4), but a graphical analysis of its behavior reveals its most

characteristic features. I will first analyze the behavior of a system in which all the intestinal transport of hexoses is carrier mediated (i.e. the passive diffusion coefficient, k_d, equals 0). Because Diamond et al (1986) have shown that hummingbirds show no passive transport of glucose, I will call this first set of models the "hummingbird model". Following this analysis I will examine the effect of a passive permeability term in the transport equation. I will call this set of models including a passive transport term the "frugivore model". The frugivorous birds studied so far do absorb glucose passively (Karasov and Levey, 1990).

Hummingbird Model

In the absence of passive transport, t_h tends asymptotically to $2V_s$ if sucrose hydrolysis is the rate limiting step ($2V_s < V_h$), and to V_h if absorption of hexoses is the rate limiting step ($2V_s > V_h$), provided that the initial concentration of sucrose is higher than the Michaelis constant of sucrase (i.e. $S_0 > k_s$, Figs 1-5a and 1-5b). This last assumption is appropriate for hummingbirds because the sucrose concentration of the food they consume is high (300 mM - 1200 mM, Baker 1975; Pyke and Waser 1981) relative to the k_m for sucrose hydrolysis (13-40 mM, Semenza 1968, chapter 4). The rate of hexose absorption from sucrose, thus, is approximately constant (i.e. follows zero order kinetics) and approximately equal to the value of the rate limiting step. V_h if absorption of hexoses is limiting and $2V_s$ if

sucrose hydrolysis is limiting. Appendix 1 provides an analytical justification for the linearity of the absorption curve in the hummingbird model.

The absorption curve of hexoses is always above the reaction/absorption curve of sucrose. If V_S is high, however, a relatively short time is required for the concentration of hexoses in the intestinal lumen to be higher than k_h, and therefore for the hexose transport system to be near saturation. Consequently, the reaction/absorption curve for sucrose converges rapidly with the hexoses absorption curve as V_S becomes larger than $V_h/2$ (see Appendix 2 for a proof, and Fig. 1-5b). For $V_S > V_h/2$ the marginal benefit derived from increasing V_S (the number of enzyme copies) tends to zero. It is difficult to predict a precise optimal ratio for V_S and V_h without an estimate of the metabolic cost of producing sucrase, the extremely small values of k_g typical of vertebrates (0.0005-0.006 μmole/μl, Karasov et al. 1985) suggest, however, that this ratio should be relatively small (Appendix 2). For example, feeding at high sucrose concentrations a hummingbird with a $k_h/V_h = 0.04$ minutes needs a V_S/V_h ratio of only 2 to achieve 96% of the maximal transport of glucose at equicaloric concentration after one minute of intestinal digestion.

Frugivore Model

I will consider first the situation of high concentration of only hexoses. Absorption kinetics are, at

least in the initial phases of absorption, of first order. As hexoses are removed from the lumen and concentration drops, the hexose absorption curve becomes decelerating and tends asymptotically to the initial concentration of hexoses (H_0, Fig. 1-5d). If the initial substrate is sucrose, the form of the reaction/absorption curves depends on the value of $2V_s$ relative to V_h. If $2V_s < V_h$, then the absorption curves are approximately linear and the rate of absorption is equal to $2V_s$. If $2V_s > V_h$ the absorption curve is sigmoidal. It increases at an accelerating rate until the rate of absorption of hexoses equals the rate of sucrose hydrolysis, becomes linear, and then decelerates when the rate of supply of hexoses provided from sucrose hydrolysis becomes smaller than the rate of hexose absorption, tending asymptotically to $2S_0$ (Fig. 5-1d, Appendix 5-1).

When V_s increases, the reaction/absorption curve for sucrose converges with the hexose absorption curve. Extremely high sucrose hydrolysis rates are required to match the initial rates of absorption that are achieved when hexoses are the substrate, however, and the ratio of V_s and V_h needed for convergence is much higher than that needed in the hummingbird model. A bird with both active and passive absorption modes and feeding on hexoses has a maximal rate of absorption at the beginning of digestion ($t = 0$) equal to $k_d H_0 + V_h$. The maximal rate of absorption that such a bird can achieve when feeding on sucrose is equal to $2V_s$ (Fig. 5-1c). For birds in which passive absorption of hexoses plays a

significant role, sucrose hydrolysis is the rate limiting step, unless $2V_s >> k_d H_0 + V_h$. This inequality becomes difficult to satisfy at the very high sugar concentrations found in most fruit pulps and nectars (Levey 1987, Lee 1970). It is more likely that a bird with a substantial passive component in the uptake of hexoses will be limited by sucrose hydrolysis, than a bird that relies only on carrier mediated uptake.

Finding the Optimal Defecation Strategy

Using the basic model developed in the previous section I will now apply static optimization techniques (reviewed in McFarland and Houston, 1981) to explore the optimal strategies of animals feeding on sucrose or monosaccharides and possessing intestines with the characteristics of the hummingbird or the frugivore models. The optimization constraints will be the kinetic parameters of hydrolysis and uptake, and the control variable will be t, the throughput time of the intestine.

The throughput time of the intestine (τ = [intestinal volume]/v_0) can be changed by altering the intestinal volume, or by modifying v_0, the rate of input to the intestine. I will only explore how changes in v_0 modify the net rate of energy intake, assuming that intestinal volume remains constant, and use the results to analyze the sugar preferences in different kinds of birds.

If intestinal volume is constant, the amount of sugar obtained from retaining a meal in the intestine τ units of time is equal to (volume) $\cdot A(\tau)$. This value can be transformed from µmolas of hexose absorbed to Joules absorbed ($E(\tau)$) by multiplying (volume) $\cdot A(\tau)$ by the energy content of sugars ($e_h = e_s/2 = 2.820$ J/µmole, CRC Handbook of Chemistry and Physics 1979). The total cost of processing a volume of intestine, $C(\tau)$, is equal to the sum of the cost of obtaining the amount of food required to fill the intestine (C_0) plus the cost of staying alive during the τ units of time required to process this amount of food. If C_m is the rate of energy expenditure of an actively digesting bird, then

$$C(\tau) = C_0 + C_m\tau \qquad (5)$$

An implicit assumption of eq (5) is that birds process discrete meals, and hence, that the cost of foraging and the gains and costs of digestion are not concurrent.

The net rate of energy gain (E/T) in the interval τ is

$$E/T = [E(\tau) - C(\tau)]/\tau \qquad (6)$$

The v_0* that maximizes this rate can be obtained by differentiating eq. (6) with respect to v_0, and setting it to zero.

$$\{\tau[(\partial E/\partial\tau)(\partial\tau/\partial v_0)-(\partial C/\partial\tau)(\partial\tau/\partial v_0)]-\partial\tau/\partial v_0(E-C)\}/\tau^2 = 0 \quad (7)$$

In "marginal value" form, E/T is maximized when τ^* is such that

$$\partial E/\partial\tau - \partial C/\partial\tau = [E(\tau) - C(\tau)]/\tau,$$

or upon further simplification, when

$$\partial E/\partial\tau = (E(\tau) - C_0)/\tau \quad (8)$$

Figure 5-2 illustrates graphically the procedure used to find τ^* and therefore v_0^* for the hummingbird model. As in many optimization models, the optimal throughput time is that in which a straight line passing through the origin is tangent to the benefit - cost curve (see Kacelnik and Houston, 1984, Sibly 1981). Note that for the particular cost function used, optimal throughput time is dependant on the cost of acquiring food C_0, but not on the rate of energy expenditure C_m.

Predictions

Hummingbird Model

Because the absorption curves are approximately linear except for very low concentrations of sugars, the optimal value of τ can be easily found and is that at which almost all the sugar is absorbed (Fig. 2-5). Thus, the model

predicts that under the conditions of the hummingbird model, birds digesting sugar meals optimally should exhibit almost 100% digestion of sugars. Throughout the text I will use the term optimal digestibility for the quantity $100X(1 - [A(t^*)/(H_0)])$ if hexoses are the substrate; or $100X(1 - [A(t^*)/(2S_0)])$ if sucrose is the substrate.

The value of t^* is always a linearly increasing function of the initial concentration of sugar (H_0 or S_0); this implies that the throughput time of animals feeding on energy-dilute foodstuffs such as dilute nectar should be lower than those of the same animals feeding on energy-concentrated foods. Because optimal energy intake rate is an increasing function of sugar concentration, animals should prefer energy concentrated to energy dilute foods.

The energy per unit mole of sucrose is about twice that of hexoses. Thus, if given a choice between foods with equiosmolar concentrations of sucrose or hexoses ($S_0 = H_0/2$), the energy maximizing birds should prefer hexoses when sucrose hydrolysis is limiting ($2V_s < V_h$), and should be indifferent if hexose absorption is limiting ($V_h < 2V_s$; Fig 5-2).

A consequence of the linearity of the cost-benefit curve of the hummingbird model is that the optimal throughput time is independent of the costs of acquiring food (C_0). The time invested in digesting a meal is independent of the cost of acquiring it.

Frugivore Model

If $2V_S < V_h$ and sucrose is the main sugar in food, then the absorption curves are approximately linear, optimal throughput time is a linearly increasing function of sucrose concentration in food and independent of the cost of acquiring food. Optimal sucrose digestibility is about 100%.

When hexoses are the main sugar in food, the maximal rate of absorption occurs at the beginning of the absorption process and decreases thereafter. Under such conditions both the cost of feeding and the concentration of hexoses of food have an effect on optimal throughput time and on optimal digestibility. Increasing the cost of feeding (C_0) increases optimal throughput times. As food becomes more expensive to acquire, it should be retained in the intestine longer and be digested more thoroughly (Fig. 5-3a).

As the concentration of hexoses in food (H_0) increases, optimal throughput time decreases. Energy rich foods should be retained in the gut a shorter time, and digested less thoroughly than energy dilute foods (Fig. 5-3b). As in the hummingbird model, optimal energy intake rate increases with sugar concentration, and animals should prefer energy concentrated to energy dilute foods.

Birds with an important passive absorption component in the intestinal uptake of hexoses, and feeding on sucrose, are more likely to be limited by hydrolysis than uptake, unless the V_S/V_h ratio is extremely high. Such birds, therefore, should prefer hexose-containing foods to foods containing

equiceloric amounts of sucrose. This preference should be more marked as the caloric concentration of food increases

Discussion

The "Hummingbird-frugivore" Dichotomy in Digestive Traits

The two models presented above are clearly extremes in a continuum, but the scant data available on the digestive physiology of nectarivorous and frugivorous birds show this clear dichotomy. The two species of hummingbirds examined by Karasov et al. (1986) show no passive intestinal permeability to glucose and extremely high rates of carrier mediated uptake of glucose. The four species of frugivores examined by Karasov and Levey (Bombycilla cedrorum, Turdus migratorius, Dumetella carolinensis and Sturnus vulgaris, Karasov and Levey 1990) exhibit relatively high passive intestinal permeabilities to glucose, and low carrier uptake rates of glucose. The dichotomy that I defined artificially, thus, may have a degree of realism. Clearly more comparative data are needed to assess its generality

Short Term Changes in Passage Rates and Sugar Digestibility

The characteristics of the fruit or nectar encountered by a foraging bird vary from day to day, and frequently from meal to meal. Fruit pulp and nectar show considerable variation in sugar concentration and composition even within a single habitat and a single plant species (Wainsworth and Wolf 1972; Levey 1987e; White and Stiles 1985, Freeman and

Worthington 1985). Pulp to seed ratios are also quite variable both within and between species of plants (Herrera 1981; Johnson et al. 1985). Frugivorous and nectarivorous birds can potentially increase their rate of energy intake by varying the time that food spends in the intestine in accordance with the chemical characteristics of food and, at least in the case of birds with high passive transport of hexoses, with the cost of acquiring it. There are few data available to examine the hypothesis that birds are able to modify their throughput times in response to short term changes in food characteristics. Evidence from other groups, however, suggests that animals do exhibit short term changes in gut throughput times in response to variations in food characteristics (see Van Soest 1983, pp. 222, Bjorndal 1989). Hummingbirds show increased throughput times and increased intervals between meals in response to increased sugar concentration in nectar, but appear to keep meal size constant (Gass 1978; Wolf and Hainsworth 1977, Martinez del Rio unpubl. data). Thus in hummingbirds the time required to process a meal appears to increase with its concentration.

A change in the time that a meal stays in the intestine can result in changes in the extent of digestion and absorption that takes place (Van Soest 1983). The model outlined above suggests that birds relying mostly on active transport of hexoses should vary retention time in accordance to the concentration of food and exhibit little variation in the efficiency with which they digest sugars, they should

always exhibit digestive efficiencies that are close to 100%. Birds with an important passive component in the uptake of hexoses, in contrast, should show considerable variation in digestibility of sugars, and this variation should be correlated with the characteristics of food. Hummingbirds show proverbially high digestive efficiencies of sucrose, approaching 100% (Hainsworth 1974, Karasov et al. 1986), and equivalently high digestive efficiencies of the hexoses glucose and fructose (chapter 3). The hypothesis that hummingbirds should exhibit digestibilities of sugars close to a 100% is crucially dependent on hummingbirds having very low passive intestinal permeabilities to hexoses. Karasov et al. (1986) speculated that low passive intestinal permeabilities were advantageous in the face of rapid fluid transit, because they would impede the loss of solutes from the blood-stream into the intestinal lumen. The relative impermeability of the intestinal membranes could also prevent osmotic water flow from the blood-stream into the intestinal lumen resulting from feeding on concentrated solutions. The osmotical loss of blood fluid into the intestine could have severe consequences in a small animal (Hunt and Knox 1968).

The frugivores in which the digestibility of sugars has been examined do not show 100% efficiencies. The digestibilities of glucose in cedar waxwings and American robins were 92% and 73% respectively (chapter 3, Karasov and Levey 1990). The assimilation efficiency of reducing sugars in free-living cedar waxwings was estimated to be about 66%

by Studier et al. (1988) In a recent review, Karasov (1985) found that the digestive efficiencies of frugivores were lower than those predicted assuming that frugivores digested all the nutrients in fruit except the refractory and cell wall fractions Because a large fraction of the non-refractory solids in fruit are sugars, it is probably correct to assert that frugivores show digestive efficiencies of sugers that are lower than 100%

Food Quality and Throughput Times

The terms "poor food" and "food of low quality" are ambiguous descriptors. They can mean that food is energy-dilute or that it takes a long time to digest and absorb Consider a bird with no passive absorption of hexoses; for such a bird sucrose is a low quality food if the ratio of rates of hydrolysis to absorption (V_s/V_h) is low, and the optimal throughput time for sucrose is in consequence relatively long In the same bird, dilute hexose-rich nectars and fruits with low pulp/seed ratios are also low quality foods, but the optimal throughput times are relatively short. The assertion that animals feeding on "low quality" food should have long throughput times (Sibly 1981), thus, does not appear to be true in general (see Calow 1975) Food quality and digestibility are a function of the interaction between a food's nutrient content and density and the digestive traits of the animal consuming it (Moss 1983)

On Measuring Intestinal Throughput Times

An important cautionary note is that for adequate testing, the predictions of the model presented above require the measurement of intestinal throughput times. These are rarely measured, and are not equal to the much more frequently measured mouth-cloaca throughput times. Total mouth-cloaca throughput times are an additive function of both the throughput times of the crop and stomach and the intestine. In some species with large crops, intestinal throughput times are likely to be short compared with the throughput time of the crop and stomach. Little is known of the relative contribution of each part of the gut to the total throughput time but it is likely that interspecific comparisons of total throughput times compare storage capacity rather than the time an average food particle spends in contact with digestive and absorptive surfaces. In the hummingbirds studied by Kerasov et al. (1986), about 13% of the mean time required to process a meal could be accounted for by the time that the meal stayed in the crop. We know little of the amount of time that food stays in the stomach of hummingbirds or other birds before it passes to the intestine. In the absence of detailed distributions of time spent in the gut by inert markers, the time required for an inert marker to appear in the excreta after ingestion ("transit time") is probably the best estimator of intestinal throughput time (Penry and Jumars 1986).

Chemical engineers commonly explore reactor design and properties by studying residence times of chemically inert markers that pass unaltered through reactors (Froment and Bischoff 1979). Residence times distributed as negative exponentials with transit times equal to 0 are typical of completely stirred tank reactors (CSTR, Levenspiel 1972). Residence times in coupled CSTR-PFR reactor are distributed as negative exponentials shifted to the right (i.e. transit times > 0, Penry and Jumars, 1986). In these systems distribution transit time is equal to the throughput time of the PFR, and the shifted negative exponential is equal to the distribution of retention time of markers in the coupled CSTR. The residence times of inert markers in the digestive tract of hummingbirds, a frugivore (cedar waxwing), and two facultative frugivores (American robin and starling) are distributed as negative exponential density functions with a very short transit time (Karasov et al 1985; Karasov and Levey 1990). Available data lack resolution at the lower end (t = 0) of the retention time distribution function and do not permit a clear diagnosis. Detailed distributions of marker retention times for birds are needed to test the predictions of the model presented above, as well as to substantiate the assumption that the intestine of insectivorous and frugivorous birds behaves as a plug-flow reactor.

Digestive Traits and Sugar Preferences

The model predicts that the preferences of birds for sugars are mediated by the interaction between the sugar composition and concentration of food, and the kinetic parameters of hydrolysis and absorption. Below I review a few studies where the preferences of birds for simple sugars were correlated with digestive traits.

An extreme example of a physiological constraint on sugar preferences can be found in European starlings. Starlings reject sucrose solutions but strongly prefer hexose solutions over water (Schuler 1983, chapter 1). Also, after being fed sucrose solutions, starlings developed a conditioned aversion (chapter 1). Martinez del Rio and Stevens (1989) demonstrated that starlings lack sucrase activity ($V_S = 0$). For birds lacking sucrase activity, sucrose is not only a useless energy source, but can cause osmotic diarrhea and act as a feeding deterrent. Starlings are mainly insectivorous but consume large quantities of hexose-dominated fruits like cherries and grapes seasonally (Martin et al. 1951). Karasov and Levey (1990) report that American robins and gray catbirds are also ineffective at absorbing sucrose, presumably as a result of the absence of sucrase activity.

Cedar waxwings show intestinal sucrase activity but prefer glucose and fructose over sucrose (chapter 2). Cedar waxwings absorb 92% and 88% of the glucose and fructose they consume, but only 60% of the sucrose (chapter 2). In cedar

waxwings the maximal rate of glucose uptake is greater than the maximal rate of sucrose hydrolysis, and cedar waxwings show substantial passive uptake of glucose (Karasov and Levey 1990). It appears, thus, that sucrose hydrolysis is the limiting step, supporting the predictions of the model. The rate of fructose uptake was not measured in cedar waxwings but it is probably similar, or slightly lower, than the rate of uptake of glucose (if cedar waxwings absorb fructose like mammals, Sigrist-Nelson and Hopper 1974, Holdsworth and Dawson 1964). The uptake mechanism of the monosaccharide fructose is virtually unknown in birds, however, and deserves more attention (Levaille et al. 1970).

The preferences of hummingbirds for different sugars were reviewed in chapter 3. All the species tested preferred sucrose over fructose, glucose, and mixtures of glucose and fructose. In spite of many species showing a marked preference for sucrose, hummingbirds appear to derive no energetic advantages from feeding preferentially on sucrose over 1:1 mixtures of glucose and fructose (chapter 3). The preference of hummingbirds for sucrose over mixtures of hexoses is puzzling. As predicted by the model, hummingbirds consistently prefer concentrated over dilute solutions (Hainsworth and Wolf 1976, Stiles 1976).

Because most hummingbirds feed on nectar containing high concentrations of sucrose, and appear to prefer sucrose over glucose and fructose, it can be predicted that they will exhibit high intestinal sucrase activities and that

absorption rather than hydrolysis will be the limiting step (i.e. that $2V_s >> V_h$). The rate of glucose absorption of the hummingbird intestine is the highest reported among vertebrates (Karasov et al. 1986); sucrase activities are also extremely high (chapter 4).

Digestive Traits and the Rewards That Plants Offer to Avian Pollinators and Seed Dispersers

The nectar secreted by hummingbird-pollinated plants is predominantly sucrose-rich or sucrose-dominant, whereas passerine pollinated flowers secrete nectars that are hexose-dominant (Baker and Baker 1983). The robustness of this dichotomy in the chemical composition of nectars is illustrated by the species of Erythrina (Fabaceae), where Old World and New World passerine-pollinated species show clear hexose dominance, and hummingbird-pollinated plants show high sucrose-hexose ratios (0.557 -2.187 in hummingbird-pollinated species, 0.015 - 0.074 in perching bird-pollinated species, from Baker and Baker 1982, 1983). Scogin and Freeman (1984) have described a similar pattern in the Andean genus Puya (Bromeliaceae). In an extensive review of the sugar composition of fruit pulps (> 200 spp.), Baker and Baker (1986, and unpubl. data) found that most bird-dispersed fruits are hexose dominant with very low sucrose/hexose ratios, whereas cultivated fruits used for human consumption have considerably higher sucrose/hexose ratios.

What could explain this pattern? Because flowers and fruit are "prey that went to be eaten", the rewards that plants offer should be accessible and easily digestible by potential pollinators and seed dispersers (Heinrich 1976, Snow 1981b). Baker and Hurd (1968) and Howell (1974) have suggested that the chemical characteristics of the rewards that plants offer reflect the preferences of pollinators and seed dispersers. I have argued that these preferences may be strongly influenced by digestive traits. One can argue that the preferences of pollinators and seed dispersers act as selective forces maintaining the preferred sugar composition of the plants that they feed on. This argument leads to a series of predictions correlating sugar preferences and digestive traits of birds with the sugar composition of the rewards that plants offer to them. Thus, most hummingbirds should prefer sucrose over hexoses, whereas flower-visiting perching birds and avian frugivores should prefer hexoses over sucrose. The model detailed above associates some physiological traits with the sugar preferences of birds. I hypothesize that among birds, frugivorous and non-hovering flower visitors will show preference for hexoses over sucrose. I also predict that this preference will be correlated with sucrose hydrolysis being a rate limiting step in the absorption of hexoses from sucrose. Hummingbirds, in contrast, should have uptake of hexoses as the rate limiting step. My model predicts that hummingbirds derive no energetic advantage from feeding preferentially on sucrose. Alternative

explanations are needed to account for the apparently widespread preference of hummingbirds for sucrose over hexoses.

The existance of a clear dichotomy in the kinds of sugars consumed by ecologically contresting groups of birds, offers a good opportunity to examine the importance of relatively subtle differences in the chemical composition of foodstuffs to the feeding preferences of animals. In a similier fashion, the examination of the digestive strategies of frugivorous and nectarivorous birds can help to understand how digestive traits can influence foraging behavior, and may help to shed some light on the possible factors that have influenced the evolution of the interaction of birds and their plant mutualists. An evolutionary explanation of the puzzling patterns of sugar distribution in the rewards that plants offer to their avian mutualists will probably have digestive physiology as an important ingredient.

Table 5-1 - Symbology and units

Symbol	Definition	units
F	fructose concentration per unit volume of digesta	µmoles/µl
G	glucose concentration per unit volume of digesta	µmoles/µl
H	hexoses (fructose + glucose) concentration per unit volume of digesta	µmoles/µl
S	sucrose concentration per unit volume of digesta	µmoles/µl
A	amount of hexoses absorbed per unit volume of digesta	µmoles/µl
E	energy uptake	Joules
C	energetic costs	Joules
C_0	cost of filling the intestine with food	Joules

C_m	rate of energy expenditure while digesting	Joules/min
r_f	fructose intestinal uptake rate	µmoles/(µl min)
r_g	glucose intestinal uptake rate	µmoles/(µl min)
r_h	hexoses (glucose+ fructose) intestinal uptake rate	µmoles/(µl min)
r_s	sucrose hydrolysis rate	µmoles/(µl min)
k_g	Michaelis constant of glucose intestinal uptake	µmoles/µl
k_h	Michaelis constant of hexose intestinal uptake	µmoles/µl
k_s	Michaelis constant of sucrase intestinal hydrolysis	µmoles/µl
k_d	Intestinal passive permeability coefficient of hexoses	min^{-1}
V_g	maximal rate (V_{max}) of glucose intestinal uptake	µmoles/(µl min)

V_h	maximal rate (V_{max}) of hexoses intestinal uptake	µmoles/(µl·min)
V_s	maximal rate (V_{max}) of sucrose intestinal hydrolysis	µmoles/(µl·min)
v_0	rate of intestinal flow of digesta	µl/min
τ	intestinal throughput time	min
τ^*	optimal intestinal throughput time	min
e_h	energy value per µmole of hexoses	Joules/µmole

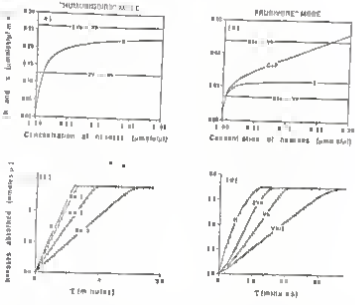

Fig. 5-1. Absorption kinetics of hexoses for the hummingbird and frugivore model. The absorption curves as a function of hexose concentration are the increasing curves shown in graphs a and c. Curve C is the absorption of hexoses resulting exclusively from carrier mediated processes; curve C + P is the sum of carrier mediated transport and passive diffusion of hexoses. Rates of sucrose hydrolysis (r_s) for different conditions are schematically shown as lines parallel to the concentration axis. Note that the concentration of hexoses achieves a transient steady state at the point where the absorption curve intersects the hydrolysis line ($dH/dt = r_a - r_g = 0$). Figures b and d show numerical solutions to eq. 4 for both models. The curve labeled H is the absorption curve for hexoses as only substrates, the remaining curves are hexose absorption curves for sucrose as the substrate and various values of V_s relative to V_h, the kinetic parameters of absorption and hydrolysis used in the hummingbird modal were $V_h = 0.24$ μmoles (µl min)$^{-1}$, $k_h = 0.004$ μmoles/µl, $k_s = 0.016$ μmoles/µl and $H_0 = 2S_0 = 1.4$ μmoles/µl. These values are similar to those of a hummingbird weighing ca. 3.5 g. In the frugivore model V_h=0.012 μmoles (µl min)$^{-1}$, $k_h = 0.004$ μmoles/µl, $k_s = 0.016$ μmole/µl, $k_d = 0.07$ min^{-1} and $H_0 = 2S_0 = 0.7$ μmoles/µl. These values resemble those of a frugivore weighing ca. 30 g. The values were chosen only for descriptive purposes.

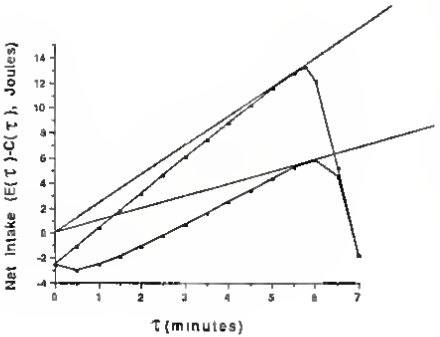

Fig. 5-2 - Optimal throughput times for a 0.7 μmoles/μl sucrose solution in the hummingbird model. Each curve represents different values of V_s ($V_s = 5V_h$ in the curve above, and $V_s = V_h/2$ in the lower curve). The kinetic values of the parameters are those of Fig. 1-5a. An intestinal volume of 20 μl was assumed. Optimal throughput times are those in which a line passing through the origin is tangent to the cost-benefit curve. The metabolic costs were calculated from Hainsworth (1978) for a resting 3.5 g hummingbird, feeding costs were estimated assuming that the bird spent 2 seconds hovering to aquire enough nectar to fill the intestine, and using the hovering costs measured by Bartholomew and Lighton (1986).

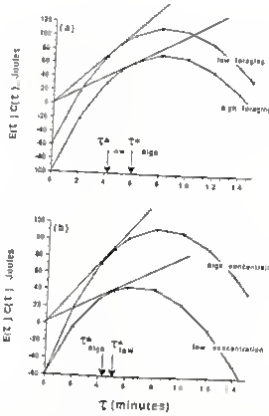

Fig 5-3 - Effect of feeding costs and sugar concentration on optimal throughput times of the frugivore model Graph (a) shows the consequences of increasing the cost of filling the intestine (C_0) on the throughput time that maximizes energy intake Graph (b) illustrates the effect of increasing food concentration from 0 6 M to 0 7 M on the optimal throughput time To calculate the cost-benefit curve I used the kinetic parameters of curve 1c, an intestinal volume of 600 µl and metabolic costs calculated for a 30 g bird from the equation 1 8 x 6 8 (M X 10^{-3})$^{0\ 704}$, W (Whittow 1986)

CHAPTER 6
CONCLUSIONS AND SPECULATIONS: BIRD PREFERENCES AND THE SUGAR CONSTITUENTS OF FLORAL NECTAR AND FRUIT PULP

The evolution of the diversity of traits in flowers and fruits and its obvious relationship to pollination and seed dispersal has been studied for over a hundred years (Baker, 1979). The forces and constraints that mold and maintain the array of floral and fruit characteristics, however, have received comparatively little attention (see Sections I and II in Jones and Little, 1983). Researchers have recognized that characteristics such as morphology, color, and smell can associate flowers and fruits with specific groups of pollinators and seed dispersers (Faegri and van der Pijl, 1966), but have rarely tried to explain why or how unrelated plants have evolved convergent syndromes in response to common groups of pollinators and dispersers.

Flowers and fruit are "prey that want to be eaten" (Heinrich, 1975), therefore, ecologists generally assume that their characteristics are a consequence of pollinator's or disperser's choice (Howell, 1979). Plants pollinated or dispersed by different groups of animals offer different kinds and concentrations of sugars, amino acids, and lipids (Baker, 1975; Baker and Baker, 1973, McKey 1975). The chemical composition of the rewards that plants offer is a good example of the diversity usually assumed to be molded by

the preferences of animals (Calder, 1979; Howell, 1979). Throughout this dissertation I have focused on the sugar constituents of nectar and fruit to examine this assumption and to try to resolve why plants offer different rewards to different kinds of animals.

To examine the relationship between the sugar constituents in nectar and fruit pulp and the preferences of birds, I adopted a multi-level approach that is widely used in community ecology (Brown et al., 1986), but rarely used to study animal-plant interactions. I identified an ecological pattern (the convergence in sugar composition in the rewards offered by plants with common pollinators or dispersers), postulated and empirically examined a process (the variation in sugar preferences among birds), and investigated the physiological and ethological mechanisms responsible for this process (Fig. 6-1). My research illustrates how examining an ecological phenomenon at many levels can reveal a good deal of the complexities that are missed when only one level of the hierarchy is examined.

At the outset of my research I used as a working hypothesis the widely held belief that the chemical composition of the rewards that plants offer is a result of an evolutionary adjustment to the preferences of pollinators and seed-dispersers (Baker and Hurd 1968; Howell 1979, Stiles 1981; Baker and Baker 1983 among others). The sugar preferences of birds supported this view. Birds appear to prefer the kinds of sugars present in the nectars and fruit

that they feed on. Analysis at this behavioral level only would have led me to accept the one-dimensional view that the main evolutionary response is made by plants.

The physiological and ethological correlates of sugar preferences in birds, however, revealed a more complex relationship and posed a new set of questions. In this chapter I will develop what I believe are plausible scenarios for the evolution of the diversity of sugar constituents in nectar and fruit pulp. These scenarios will serve to frame the many questions and hypotheses posed in the previous chapters. I will first deal with the evolution of the sugar constituents in nectar and the vexing question of the sucrose preferences of hummingbirds, then, I will tackle the evolution of hexose preferences in fruit-eating birds and hexose dominance in fruit-pulp. In a final section I will point at the limitations of the multi-level approach that guided my research as a tool to provide evolutionary explanation for traits--such as the composition of sugar in nectar and fruit--that may be the result of historical coevolutionary processes.

Nectar Composition and the Sugar Preferences of Birds

By virtually every criterion--be it morphological, behavioral or physiological--hummingbirds are the most specialized nectar-feeding birds and show several differences when compared with passerine nectarivores (see table 3 in Stiles 1981, and Collins and Paton 1989, and Paton and

Collins 1989). I hypothesize that hummingbirds and most nectar-feeding passerines differ in digestive abilities as well. Specifically, I suggest that the digestive physiology of hummingbirds is well suited for a sucrose diet whereas the digestive physiology of nectar-feeding passerines is not (with the possible exception of species such as Diglossa spp. and Coereba flaveola, which sometimes feed parasitically on the sucrose-dominated nectar of hummingbird-pollinated plants, Lyon and Chadek, 1971; Stiles, 1981).

Data on the few New World passerine nectar-feeding species that have been examined support the above hypothesis. Two out of three species showed a preference for a hexose mixture over sucrose and assimilated sucrose inefficiently (Martinez del Rio unpubl. data). The digestive traits and sugar preferences of Old World nectarivores are unknown, but I predict that they prefer hexoses over sucrose and that sucrose will be assimilated poorly or more slowly than glucose and fructose. To my knowledge no data are available to falsify this hypothesis. Collins and Morellini (1979) and Collins et al. (1980) reported that sucrose assimilation was extremely high in two honeyeater species (Meliphaga virescens, and Lichmera indistincta, Meliphagidae) but did not compare the rate of sucrose processing with that of hexoses. Clearly, more data are needed on the sugar preferences and digestive abilities of Old World nectar-feeding birds.

At the outset of my research I hypothesized that the preferences of birds are the selective pressure responsible

for maintaining the sugar composition in the rewards that plants offer, and that differences in digestive handling can explain the variation in preferences among different groups of birds. The evidence suggests, however, that digestive mechanisms do not explain the preferences of hummingbirds for sucrose over hexose mixtures (see chapter 3). Why some hummingbird species prefer sucrose over hexoses is a vexing question that has no satisfactory answer. It is especially vexing because answering it may help to answer the related question of why do hummingbird-pollinated plants secrete sucrose instead of glucose and fructose?

In chapter 3 I suggest a possible answer to this question. I argue that sucrose is the major form in which energy is translocated in most plant species (Hawker, 1985), and that it is probably cheaper for plants to secrete sucrose directly from the phloem than to hydrolyze it in the nectaries into glucose and fructose (Fahn, 1979). It is likely that in the absence of pollinator selection against sucrose, sucrose producing phenotypes are advantageous. The digestive adaptations of hummingbirds allow them to use sucrose as efficiently as hexose mixtures, and in consequence hummingbird-pollinated plants are allowed to secrete sucrose dominated nectar. Passerine-pollinated plants, in contrast, seem to be visited and pollinated by a diverse group of relatively unspecialized species, some of which cannot use sucrose as efficiently as they can the readily assimilable monosaccharides glucose and fructose. In these plants the

presence of sucrose in nectar would act as a floral filter excluding potential pollinators

The distribution of floral nectar constituents in bird-pollinated plants appears to be the result of the evolutionary interaction between plants and two sets of pollinators with contrasting degrees of digestive specialization (chapter 3). This scenario explains well the divergent evolution of the two bird pollination syndromes in the New World, where passerine pollination is of more recent origin than hummingbird pollination (Stiles 1981), and where most passerine flower visitors are facultative and without doubt less specialized than hummingbirds (Cruden and Toledo, 1977; Gryj, 1980).

In Australasia, Africa, and Hawaii, several families of passerines have independently evolved sophisticated morphological specializations that enhance the ability to feed on nectar (Collins and Paton, 1989). My scenario for the evolution of nectar sugar composition suggests, however, that they have not evolved the sucrose-adapted digestive physiology of hummingbirds. The existence of specialized nectar-feeding birds in the Old World poses a puzzling question: why did the digestive traits of hummingbirds followed a divergent path from those of the Old World nectarivorous birds?

At the moment I do not have a convincing answer. I suspect, however, that the contrasting pollination spectra of the flores with which hummingbirds and passerines evolved may

hold the key to this question. Hummingbird-pollinated flowers are derived mostly from flowers pollinated by butterflies and large bees (Grant and Grant, 1968, Stiles, 1981), both of which secrete sucrose-rich or sucrose-dominant nectars (Baker and Baker, 1983). Presumably, proto-hummingbirds had to evolve the digestive adaptations to feed on sucrose before they became the "most effective pollinators" (sensu Stebbins 1970) of these plants. Large flower-visiting bees are scarcer in Australia and Africa than in the New World (Michener 1970, Baker, 1973). In the Old World, bird-pollinated flowers are derived from a variety of other syndromes, including small-bee, fly and mammal pollination (Ford et al., 1979). Flowers in these syndromes tend to secrete nectars with lower sucrose/hexose ratios than large bee and butterfly flowers (Baker and Baker, 1983). It may be that hummingbirds and Old World nectar-feeding passerines inherited floras with contrasting pollination spectra, and that in consequence, they now occupy different digestive "adaptive zones" (sensu Simpson 1949).

Seed Dispersal and the Chemical Composition of Fruit-pulp

I have identified two mechanisms that can account for the preference for hexoses over sucrose in frugivorous birds: fast passage rates and lack of intestinal sucrase. In this section I explore the causes and consequences of the first of these mechanisms.

Fruits are the vehicles used by plants for seed dispersal and are by necessity bulky. The juicy fruits that are typically dispersed by small passerine birds are characterized by a nutrient-dilute pulp and a large proportion of seeds (Moermond and Denslow, 1985). The presence of seeds in fruit reduces total gut capacity and imposes a heavy undigestible load on flying fruit-feeding dispersers (Levey, 1987). Levey and Grajal (1990) have argued that the fast gut passage rates typical of frugivores evolved as a mechanism to void the ballast that undigestible seeds represent. Indeed, rapid processing of seeds and pulp seems to be a trait common to most, if not all, small fragivores (Morrison, 1980, Harrara 1984).

Apparently fast passage rates do not provide the necessary processing time for complex substrates such as sucrose, which must be hydrolyzed to be assimilated, and hence impose a severe digestive constraint on frugivorous birds (chapter 2). Levey and Grajal (1990) argue that frugivores may have selected for nutrients in fruit pulp that can be rapidly assimilated, such as monosaccharides and free amino acids, and against complex nutrients that require digestion prior to absorption such as sucrose, polysaccharides, and polypeptides (Milton, 1981, chapter 2).

The low sucrose/hexose ratios found in the fruit pulp of bird-dispersed fruits are probably the result of benefits to birds of rapid disposal of seeds. Fast passage rates, therefore, may causally link frugivory and seed dispersal

with the preferance of bird frugivoras for glucose and fructose. Tha absence of sucrose--and praaumably other complex substrates--in fruit pulps appaara to be the result of diffuse coevolution (senau Futuyma and Slatkin 1983) between birds and plants, and to be mediated by the digestive adaptations of birds to a frait diet (Fig 6-2)

Lack of Sucrase: A Phylogenetic Singularity With Ecological Consequences?

In a sample of 22 species, lack of intestinal sucrase activlty appeared to be restricted to thrushas (Muscicapidae), starlings (Sturnidae), and thrashers (Mimidae, chapter 4). Theae three families are members of a single phylogenetic lineage (Siblay and Ahlquiat,1984; Sibley et al., 1988) that includes many nectarivorous and frugivorous species, some of which are quite specialized (e.g. Promerops, Olson and Ames 1984). The cosmopolitan family Muscicapidae contains the largest number of seed-dispersing genera and spacies in the Nearctic and Palearctic realms (Herrera, 1984; Willson, 1986; Snow and Snow, 1988). The families Mimidae and Sturnidae also include many highly frugivorous spacies in the New and the Old World (Snow, 1981; Willson, 1986).

In praaant-day communitias, the preferences of sucrose-intolarant species may ba a strong selective force thet contributes to the maintanance of low sucrose concentrations in nactar and fruit pulp. Lack of intestinal sucrase,

however, is an unlikely explanation for the origin of hexose dominance in fruit pulp. Fruit-eating appeared very early in the evolutionary history of birds (Regal, 1977). Fossil trogons (Trogonidae) and touracos (Musophagidae) can be found in Eocene and early Oligocene deposits (Olson 1985). The sturnid-muscicapid lineage probably appeared after the Miocene (Olson 1985, Sibley and Ahlquist 1984). Thus, frugivory and its coevolutionary correlates certainly antedated the evolution of the sturnid-muscicapid lineage, making it difficult to argue that hexose dominance in fruit-pulp is a result of the selective pressure exerted by sucrose-intolerant birds. It is more likely that hexose dominance in fruit pulp originally evolved in response to fast passage rates, and subsequently permitted the development of frugivory in the asucrotic sturnid-muscicapid lineage, facilitating a very successful frugivorous radiation (Fig. 6-3).

The Role of Historical Singularities

Gilbert and Raven (1973) have pointed out the difficulty of integrating ecological and evolutionary time in coevolutionary studies. Coevolutionary relationships are by definition the product of historical change, yet this historical change is still proceeding (Gilbert and Raven 1973). The multi-level approach that I have used to guide my work emphasizes ecological time and therefore includes an implicit equilibrium assumption; it assumes that current

mechanisms and processes are responsible for **maintaining** a given coevolutionary pattern. The results of history, however, rarely specify their initial causes unambiguously (Gould 1980). In consequence, my chosen approach does not provide many insights on the **origins** of the mechanisms and processes that underly the peculiar distribution of the sugar constituents of nectar and fruit pulp.

In particular my approach fails to provide explanations to two vexing questions. Why do hummingbirds have digestive physiologies that are sucrose adapted, whereas passerine nectarivores apparently do not, and why do starlings and robins lack intestinal sucrase activity? Using biogeographical and phylogenetic data I have suggested preliminary explanations. I have argued that perhaps the dichotomy in the digestive traits of hummingbirds and nectarivorous passerines is the result of coevolution between these groups and floras with contrasting pollination spectra. I also have argued that the lack of sucrase in Starlings and Robins is a trait associated with a single phylogenetic group, the sturnid-muscicapid lineage (Sibley et al., 1988). Lack of sucrase may be a good example of a phylogenetic constraint with clear behavioral and ecological consequences. The multi-level approach that I chose to study the sugar constituents in nectar and fruit pulp is not in itself sufficient to provide adequate evolutionary explanations. An adequate evolutionary explanation for the distribution of sugar constituents in the nectar and fruit-pulp of

ornithophylous plants will require integrating nectar chemistry with bird behavior and physiology in a biogeographic and phylogenetic perspective.

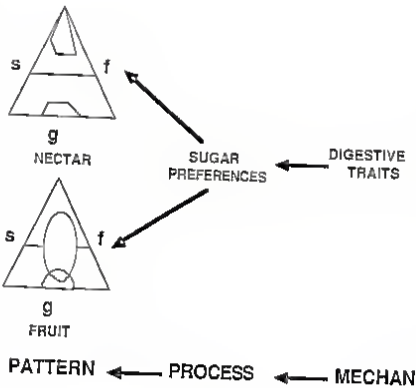

Fig 6-1 - Diagramatic representation of the multi-level approach that guided my research. The triangular charts show the distribution of sucrose (s), glucose (g), and fructose proportions in nectar (upper triangle) and fruit pulp (lower triangle). I hypothesized that the pattern of distribution of sugar constituents in nectar and fruit pulp was maintained by a behavioral process, the sugar preferences of birds. I also hypothesized that the digestive traits of birds were the mechanisms that underlie the sugar preferences of different groups of birds.

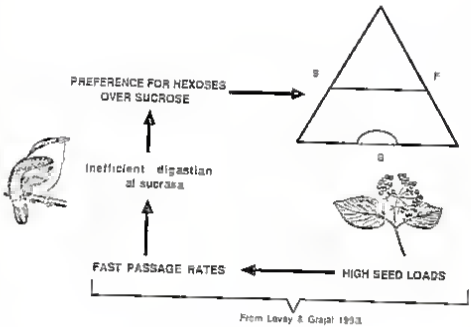

Fig. 6.2 - Hypothetical scenario for the evolution of hexose dominance in fruit pulp. The triangular chart plots the distribution of sugar constituents in the pulp of bird-dispersed fruits. I hypothesize that the low sucrose/hexose ratios found in the fruit pulp of bird-dispersed fruits are probably the evolutionary result of benefits to birds of rapid disposal of seeds.

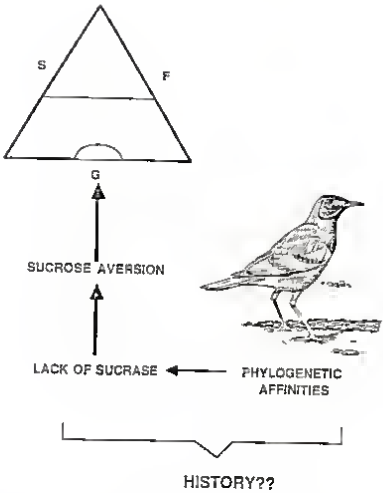

Fig. 6-3. - Hypothetical relationship between lack of intestinal sucrase activity in the sturnid-muscicapid lineage and the sugar constituents in fruit pulp. I argue that lack of sucrase in this lineage is one of the selective forces that contributes to **maintain** hexose-dominance in fruit pulps. I hypothesize that **the origin** of the lack of sucrase activity in the sturnid-muscicapid lineage is unrelated to the sugar composition of fruit and probably the result of a historical singularity.

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APPENDIX 4-1

Appendix 4-1.- Body mass, summed sucrase and maltase activity measured at 28 mM substrate concentration in three species of hummingbirds and eleven species of passerine birds. Values are means ± SE and n is number of individuals.

	body mass (grams)	summed sucrase activity (μmoles/min)	summed maltase activity (μmole/min)	N
Trochilidae				
Chlorostilbon canivetii	2.1 ± .1	4.48 ± .36	8.49 ± .27	4
Cynanthus latirostris	2.9 ± .1	5.56 ± .45	14.02 ± 1.3	5
Amazilia rutila	4.5 ± .6	8.26 ± .90	20.75 ± 3.51	4
Tyrannidae				
Empidonax difficilis	10.9 ± 1.3	33 ± .12	7.15 ± 2.51	2
Pitangus sulphuratus	73.3 ± 5.7	6.94 ± 1.01	39.91 ± 6.20	3
Myiozetetes similis	28.9 ± 2.6	3.05 ± .47	29.74 ± 4.13	3
Muscicapidae				
Catharus aurantiirostris	25.2 ± 2.0	trace [a]	5.47 ± 1.12	2
Turdus rufopalliatus	70.4 ± 5.8	trace	9.45 ± 2.15	3

Emberizidae				
Icteria virens	21.9 ± 2.0	3.33 ± .27	58.77 ± 0.80	3
Saltator coerulescens	45.6 ± 0.3	4.03 ± .37	67.39 ± .50	3
Passerina leclancherii	15.0 ± 2.1	3.83 ± .2	47.70 ± .25	2
Icterus spurius	18.5 ± 2.6	13.63 ± .46	104.38 ± 3.29	3
Icterus pustulatus	28.2 ± 4.1	8.49 ± .92	60.76 ± 6.27	3
Cacicus melanicterus	84.9 ± 18.0	18.46 ± 5.06	223.85 ± 4.50	3

[a] trace < 0.05 µmole/min

APPENDIX 4-2

Appendix 4-2.- Average sucrase and maltase activity (total activity/total intestinal nominal area), body mass and Michaelis constant of sucrase in three species of hummingbirds and eleven species of passerine birds. Values are means ± SE, n is number of individuals.

Species	average sucrase activity (μmoles/(min cm² nominal area))	average maltase activity (μmoles/(min cm² nominal area))	sucrase k_m (mM)	N
Trochilidae				
Chlorostilbon canivetii	3.88 ± .22	7.44 ± .40	38.7 ± 1.4	4
Cynanthus latirostris	3.36 ± .34	8.57 ± 1.23	30.6 ± 3.2	5
Amazilia rutila	3.91 ± .36	9.80 ± 1.57	39.8 ± 3.0	4
Tyrannidae				
Empidonax difficilis	0.07 ± .1	1.51 ± .25	4.2 ± .2	2
Pitangus sulphuratus	0.32 ± .9	1.86 ± .55	7.6 ± .1	3
Myiozetetes similis	0.28 ± .9	2.64 ± .61	8.2 ± .3	3
Muscicapidae				

Catharus aurantiirostris	trace[a]	0.60 ± .04	---	2
Turdus rufopalliatus	trace	0.39 ± .02	---	3
Emberizidae				
Icteria virens	0.31 ± .5	5.43 ± .16	12.2 ± .6	3
Saltator coerulescens	0.25 ± .8	4.10 ± .62	10.5 ± .7	3
Passerina leclancherii	0.69 ± .2	8.59 ± .25	20.5 ± .1	2
Icterus spurius	2.07 ± .11	15.89 ± .74	25.3 ± 1.0	3
Icterus pustulatus	1.16 ± .5	8.58 ± .36	17.9 ± 0.8	3
Cacicus melanicterus	0.79 ± .6	9.56 ± .50	20.1 ± 1.1	3

[a] trace < 0.005 μmole/(min. cm^2 nominal area).

APPENDIX 5-1

Appendix 5-1

The qualitative features of the reaction/absorption curve can be examined by analyzing the sign of the second derivative of $A(\tau)$:

$$d^2A/d\tau^2 = dH/d\tau\ ([k_h/(k_h + H)] + k_d)$$

The reaction/absorption curve is linear if $d^2A/d\tau^2$ equals zero. This occurs when $dH/d\tau = 0$, namely when the rate of supply of sucrose hydrolysis products is equal to the rate of uptake of hexoses. This happens when the absorption curve in Figs. 1a and 1b crosses the $2V_s$ line. If $2V_s < V_h$ this event occurs very early in the absorption process, and the reaction/absorption kinetics behave as zero order until almost all the substrate is absorbed.

In the hummingbird model the value of $d^2A/d\tau^2$ ($= dH/d\tau\ ([k_h/(k_h + H)])$ is always close to zero because the term $(k_h/(k_h + H))$ is always small ($k_d = 0.005-0.0006$ µmole/µl in vertebrates, Karasov et al. 1985). The reaction/absorption curves are therefore approximately linear until H drops below k_h.

In the frugivore model $d^2A/d\tau^2$ ($=dH/d\tau\ ([k_h/(k_h + H)] + k_d)$) is greater than zero, and therefore $A(\tau)$ is an accelerating curve, whenever the supply of sucrose hydrolysis products is larger than the uptake of hexoses ($H < (2Vs - Vh)/kd$, assuming that $2V_s > V_h$). The reaction/absorption curve becomes decelerating when $dH/d\tau < 0$. This occurs for

all t when hexoses are the sole substrate, or when the rate of sucrose hydrolysis products becomes smaller than the rate of uptake.

APPENDIX 5-2

Appendix 5-2

Here I show that in the hummingbird model as $2V_s$ increases, the reaction/absorption curve of sucrose converges with the absorption curve of hexoses.

If $S >> \{k_s, k_h\}$, then

$$dH/d\tau = 2r_s - r_h \approx 2V_s - V_h,$$

and $H \approx (2V_s - V_h)\tau$

Substituting $H = (2V_s - V_h)\tau$ in $dA/d\tau$ one gets,

$$dA/d\tau \approx V_h\{2V_s - V_h)\tau/(\tau 2V_s + (k_h - V_h\tau))$$

Because $\lim_{V_s \to \infty} \{V_h[\{2V_s - V_h)\tau/(k_h + (2V_s - V_h\tau)]\} = V_h,$

the sucrose reaction/absorption curve converges with the curve for hexoses.

Setting $V_s = nV_h$, it can be shown that $dA/dt \approx fV_h$, where f is a fraction, when

$$n = 1 + [k_h/V_h][f/(1 - f)\tau].$$

The rate at which $dA/d\tau$ tends to V_h, therefore, is governed by the value of k_h relative to the value of V_h.

BIOGRAPHICAL SKETCH

I was born in April 1956 in Mexico City and promptly taken by my young and adventurous parents to a small forestry operation at the Sierra Madre del Sur where my father worked. When I was one month old I became sick with what doctors diagnosed as polio. Moved by my mother's pain, one of my father's employees revealed his true identity. He was a medicine man ("curandero") and was going to cure me with prayer, herbs and aguardiante. He did.

I come from a lineage of inventors, ranchers, and sea-faring merchants. My great-grandfather invented a standing exercise bicycle, wrote a book on how to prevent train crashes and was the first Spaniard in Antarctica. He burned his house down trying to invent central heat. His son in law, my grandfather, was uncommon because he was a scientist but he was also wise. He went to Iceland to study geysers. Aldous Huxley was his friend and people from all political persuasions liked him, which is unusual in a country like México. He may have been interested in this dissertation.

I grew up in cattle ranches, coffee plantations and sugar cane mills. As a boy I concocted stories and told outrageously unbelievable lies. Not because I was lonely but

simply because I liked it. No one in my family has believed anything I have said ever since. I developed a strong interest in Natural History after having killed and looked at the innards of many animals. My father strongly disapproved of my interest in biology because he dislikes killing. He never discouraged me, however, and always ate what I brought to our table. He influenced me by giving me books and by being gentle.

I went to college at México's biggest university where I learned to enjoy mathematics and the hunt-game of research. It took me a long time to graduate because I consistently refused to take, or pass, courses that I didn't enjoy. After college I came to the University of Florida. Here I worked hard for the first time in my life and met Martha Wilson. Working hard almost prevented me from knowing Martha. Before coming here I never thought that I was going to get a Ph.D. or to teach anything to anybody.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Peter Feinsinger, Chair
Professor of Zoology

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This thesis was submitted to the Graduate FAculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfiliment of the requirements for the degree of Doctor of Philosophy

December 1990

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